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* * * * * * * * *   Welcome to STN International   * * * * * * * * *

 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  SEP 09  ACD predicted properties enhanced in REGISTRY/ZREGISTRY
 NEWS  4  OCT 03  MATHDI removed from STN
 NEWS  5  OCT 04  CA/CAplus-Canadian Intellectual Property Office (CIPO) added
                  to core patent offices
 NEWS  6  OCT 13  New CAS Information Use Policies Effective October 17, 2005
 NEWS  7  OCT 17  STN(R) AnaVist(TM), Version 1.01, allows the export/download
                  of CAplus documents for use in third-party analysis and
                  visualization tools
 NEWS  8  OCT 27  Free KWIC format extended in full-text databases
 NEWS  9  OCT 27  DIOGENES content streamlined
 NEWS 10  OCT 27  EPFULL enhanced with additional content
 NEWS 11  NOV 14  CA/CAplus - Expanded coverage of German academic research

 NEWS EXPRESS  NOVEMBER 18 CURRENT VERSION FOR WINDOWS IS V8.01,
               CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005.
               V8.0 USERS CAN OBTAIN THE UPGRADE TO V8.01 AT
               http://download.cas.org/express/v8.0-Discover/

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FILE 'HOME' ENTERED AT 19:42:52 ON 25 NOV 2005

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
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FILE 'USPATFULL' ENTERED AT 19:43:01 ON 25 NOV 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 24 Nov 2005 (20051124/PD)
FILE LAST UPDATED: 24 Nov 2005 (20051124/ED)
HIGHEST GRANTED PATENT NUMBER: US6968571
HIGHEST APPLICATION PUBLICATION NUMBER: US2005262612
CA INDEXING IS CURRENT THROUGH 24 Nov 2005 (20051124/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 24 Nov 2005 (20051124/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Oct 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Oct 2005

>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
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>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
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>>>  enter this cluster.                                               <<<
>>>                                                                    <<<
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>>>  classifications, or claims, that may potentially change from      <<<
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>>> the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate substance identification.

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=> e chang g j j/in
E1            2     CHANG FUNG YUEL/IN
E2            1     CHANG FWU TSAIR/IN
E3            0 --> CHANG G J J/IN
E4            1     CHANG GAN CHIEH/IN
E5            2     CHANG GAN HOW/IN
E6            1     CHANG GAO WEI/IN
E7            3     CHANG GAP SOO/IN
E8            5     CHANG GARY/IN
E9            1     CHANG GAUSS/IN
E10           1     CHANG GAVIN/IN
E11          42     CHANG GEE KUNG/IN
E12           1     CHANG GENE H/IN

=> e chang gwong j j/iin
'IIN' IS NOT A VALID EXPAND FIELD CODE FOR FILE 'USPATFULL'
The indicated field code is not available for EXPAND in this
file.  To see a list of valid EXPAND field codes, enter HELP
SFIELDS at an arrow prompt (=>).

=> e chang gwong j j/in
E1            3     CHANG GWO JER/IN
E2            3     CHANG GWO YANG/IN
E3            0 --> CHANG GWONG J J/IN
E4            2     CHANG GWONG JEN J/IN
E5            2     CHANG GYU HWAN/IN
E6            2     CHANG H J/IN
E7            2     CHANG HA Y/IN
E8            1     CHANG HAE C/IN
E9            2     CHANG HAE CHOON/IN
E10           1     CHANG HAE KWUN/IN
E11           1     CHANG HAE S/IN
E12          10     CHANG HAE SUNG/IN

=> s e4
L1            2 "CHANG GWONG JEN J"/IN

=> d l1,cbib,1-2

L1   ANSWER 1 OF 2  USPATFULL on STN
2005:188895 Nucleic acid vaccines for prevention of flavivirus infection.
    Chang, Gwong-Jen J, Fort Collins, CO, UNITED STATES
    US 2005163804 A1 20050728
    APPLICATION: US 2003-500796  A1  20020404 (10)
    WO 2002-US10764 20020404
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1   ANSWER 2 OF 2  USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
    Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
    US 2003022849 A1 20030130
    APPLICATION: US 2001-826115  A1  20010404 (9)
    PRIORITY: US 1998-87908P  19980604 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> d l1,cbib,ab,clm,1-2

L1   ANSWER 1 OF 2  USPATFULL on STN
2005:188895 Nucleic acid vaccines for prevention of flavivirus infection.
    Chang, Gwong-Jen J, Fort Collins, CO, UNITED STATES
    US 2005163804 A1 20050728
    APPLICATION: US 2003-500796  A1  20020404 (10)
    WO 2002-US10764 20020404
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      The present invention encompasses isolated nucleic acids containing
        transcriptional units which encode a signal sequence of one flavivirus
        and an immunogenic flavivirus antigen of a second flavivirus or of a
        chimeric immunogenic flavivirus antigen comprising sequence from more
        than one flavivirus. The invention further encompasses a nucleic acid
        and protein vaccine and the use of the vaccine to immunize a subject
```

against flavivirus infection. The invention also provides antigens encoded by nucleic acids of the invention, antibodies elicited in response to the antigens and use of the antigens and/or antibodies in detecting flavivirus or diagnosing flavivirus infection.

CLM What is claimed is:

1. An isolated nucleic acid comprising a transcriptional unit encoding a signal sequence of a structural protein of a first flavivirus and an immunogenic flavivirus antigen of a second flavivirus, wherein the transcriptional unit directs the synthesis of the antigen.

2. The nucleic acid of claim 1, wherein the signal sequence is a Japanese encephalitis virus signal sequence.

3. The nucleic acid of claim 1, wherein the immunogenic flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and West Nile virus.

4. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of West Nile virus.

5. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of yellow fever virus.

6. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of St. Louis encephalitis virus.

7. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of Powassan virus.

8. The nucleic acid of claim 1, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.

9. The nucleic acid of claim 8, wherein the antigen is both the M protein and the E protein of a flavivirus.

10. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

11. The nucleic acid of claim 10, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:21 and SEQ ID NO:23.

12. The nucleic acid of claim 1, wherein the transcriptional unit comprises a control sequence disposed appropriately such that it operably controls the synthesis of the antigen.

13. The nucleic acid of claim 12, wherein the control sequence is the cytomegalovirus immediate early promoter.

14. The nucleic acid of claim 1, comprising a Kozak consensus sequence located at a translational start site for a polypeptide comprising the antigen encoded by the TU.

15. The nucleic acid of claim 1 wherein the transcriptional unit comprises a poly-A terminator.

16. A cell comprising the nucleic acid of claim 1.

17. A composition comprising the nucleic acid of claim 1 and a pharmaceutically acceptable carrier.

18. A method of immunizing a subject against infection by a flavivirus, comprising administering to the subject an effective amount of the composition of claim 17.

19. The method of claim 18, wherein the flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and West Nile virus.

20. The method of claim 18, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.

21. The method of claim 20, wherein the antigen is both the M protein and the E protein of a flavivirus, and wherein a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the E protein.

22. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of West Nile virus.

23. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of yellow fever virus.

24. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of St. Louis encephalitis virus.

25. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of Powassan virus.

26. The method of claim 18, comprising administering the composition to the subject in a single dose.

27. The method of claim 18, wherein the composition is administered via a parenteral route.

28. The nucleic acid of claim 1, wherein the antigen is a St. Louis encephalitis virus antigen.

29. The method of claim 18, wherein the antigen is a St. Louis encephalitis virus antigen.

30. The nucleic acid of claim 1, wherein the antigen is a Japanese encephalitis virus antigen.

31. The method of claim 18, wherein the antigen is a Japanese encephalitis virus antigen.

32. The nucleic acid of claim 1, wherein the antigen is a yellow fever virus antigen.

33. The method of claim 18, wherein the antigen is a yellow fever virus antigen.

34. The nucleic acid of claim 1, wherein the antigen is a dengue virus antigen.

35. The method of claim 18, wherein the antigen is a dengue virus antigen.

36. The nucleic acid of claim 1, wherein the antigen is a West Nile virus antigen.

37. The method of claim 18, wherein the antigen is a West Nile virus antigen.

38. An antigen produced from the nucleic acid of claim 1.

39. A method of detecting a flavivirus antibody in a sample, comprising: (a) contacting the sample with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antibody in the sample.

40. An antibody produced in response to immunization by the antigen of claim 38.

41. A method of detecting a flavivirus antigen in a sample, comprising: (a) contacting the sample with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting

antigen/antibody complex formation, thereby detecting a flavivirus antigen in a sample.

42. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

43. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

L1 ANSWER 2 OF 2 USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
US 2003022849 A1 20030130
APPLICATION: US 2001-826115 A1 20010404 (9)
PRIORITY: US 1998-87908P 19980604 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention encompasses isolated nucleic acids containing transcriptional units which encode a signal sequence of one flavivirus and an immunogenic flavivirus antigen of a second flavivirus. The invention further encompasses a nucleic acid and protein vaccine and the use of the vaccine to immunize a subject against flavivirus infection. The invention also provides antigens encoded by nucleic acids of the invention, antibodies elicited in response to the antigens and use of the antigens and/or antibodies in detecting flavivirus or diagnosing flavivirus infection.

CLM What is claimed is:

1. An isolated nucleic acid comprising a transcriptional unit encoding a signal sequence of a structural protein of a first flavivirus and an immunogenic flavivirus antigen of a second flavivirus, wherein the transcriptional unit directs the synthesis of the antigen.

2. The nucleic acid of claim 1, wherein the signal sequence is a Japanese encephalitis virus signal sequence.

3. The nucleic acid of claim 1, wherein the immunogenic flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and West Nile virus.

4. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of West Nile virus.

5. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of yellow fever virus.

6. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of St. Louis encephalitis virus.

7. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of Powassan virus.

8. The nucleic acid of claim 1, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.

9. The nucleic acid of claim 8, wherein the antigen is both the M protein and the E protein of a flavivirus.

10. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

11. The nucleic acid of claim 10, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:21 and SEQ ID NO:23.

12. The nucleic acid of claim 1, wherein the transcriptional unit comprises a control sequence disposed appropriately such that it operably controls the synthesis of the antigen.

13. The nucleic acid of claim 12, wherein the control sequence is the cytomegalovirus immediate early promoter.

14. The nucleic acid of claim 1, comprising a Kozak consensus sequence located at a translational start site for a polypeptide comprising the antigen encoded by the TU.

15. The nucleic acid of claim 1 wherein the transcriptional unit comprises a poly-A terminator.

16. A cell comprising the nucleic acid of claim 1.

17. A composition comprising the nucleic acid of claim 1 and a pharmaceutically acceptable carrier.

18. A method of immunizing a subject against infection by a flavivirus, comprising administering to the subject an effective amount of the composition of claim 17.

19. The method of claim 18, wherein the flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and West Nile virus.

20. The method of claim 18, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.

21. The method of claim 20, wherein the antigen is both the M protein and the E protein of a flavivirus, and wherein a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the E protein.

22. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of West Nile virus.

23. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of yellow fever virus.

24. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of St. Louis encephalitis virus.

25. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of Powassan virus.

26. The method of claim 18, comprising administering the composition to the subject in a single dose.

27. The method of claim 18, wherein the composition is administered via a parenteral route.

28. The nucleic acid of claim 1, wherein the antigen is a St. Louis encephalitis virus antigen.

29. The method of claim 18, wherein the antigen is a St. Louis encephalitis virus antigen.

30. The nucleic acid of claim 1, wherein the antigen is a Japanese encephalitis virus antigen.

31. The method of claim 18, wherein the antigen is a Japanese encephalitis virus antigen.

32. The nucleic acid of claim 1, wherein the antigen is a yellow fever virus antigen.

33. The method of claim 18, wherein the antigen is a yellow fever virus antigen.

34. The nucleic acid of claim 1, wherein the antigen is a dengue virus antigen.

35. The method of claim 18, wherein the antigen is a dengue virus antigen.

36. The nucleic acid of claim 1, wherein the antigen is a West Nile virus antigen.

37. The method of claim 18, wherein the antigen is a West Nile virus antigen.

38. An antigen produced from the nucleic acid of claim 1.

39. A method of detecting a flavivirus antibody in a sample, comprising: (a) contacting the sample with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antibody in the sample.

40. An antibody produced in response to immunization by the antigen of claim 38.

41. A method of detecting a flavivirus antigen in a sample, comprising: (a) contacting the sample with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antigen in a sample.

42. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

43. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

```
=> s (JEV or japanese encephalitis virus)
          121 JEV
       411012 JAPANESE
         7955 ENCEPHALITIS
        94470 VIRUS
          805 JAPANESE ENCEPHALITIS VIRUS
                (JAPANESE(W)ENCEPHALITIS(W)VIRUS)
L2        825 (JEV OR JAPANESE ENCEPHALITIS VIRUS)

=> s l2 and (signal sequence)
      1293871 SIGNAL
       750754 SEQUENCE
        27677 SIGNAL SEQUENCE
                (SIGNAL(W)SEQUENCE)
L3        144 L2 AND (SIGNAL SEQUENCE)

=> s l3 and ay<2002
      3495426 AY<2002
L4         80 L3 AND AY<2002

=> s l4 and signal/clm
       642050 SIGNAL/CLM
L5         19 L4 AND SIGNAL/CLM

=> d l5,ti,1-19

L5   ANSWER 1 OF 19  USPATFULL on STN
TI     Chimeric flavivirus vaccines

L5   ANSWER 2 OF 19  USPATFULL on STN
TI     Non-stochastic generation of genetic vaccines and enzymes

L5   ANSWER 3 OF 19  USPATFULL on STN
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TI Chimeric flavivirus vaccines

L5 ANSWER 4 OF 19 USPATFULL on STN
TI Novel co-stimulatory molecules

L5 ANSWER 5 OF 19 USPATFULL on STN
TI Novel co-stimulatory molecules

L5 ANSWER 6 OF 19 USPATFULL on STN
TI Non-A, non-B, non-C, non-D, non-E hepatitis reagents and methods for their use

L5 ANSWER 7 OF 19 USPATFULL on STN
TI Nucleic acid vaccines for prevention of flavivirus infection

L5 ANSWER 8 OF 19 USPATFULL on STN
TI Non-stochastic generation of genetic vaccines

L5 ANSWER 9 OF 19 USPATFULL on STN
TI Novel chimeric promoters

L5 ANSWER 10 OF 19 USPATFULL on STN
TI Immunological combination compositions and methods

L5 ANSWER 11 OF 19 USPATFULL on STN
TI Immunological combination compositions and methods

L5 ANSWER 12 OF 19 USPATFULL on STN
TI Methods of preparing carboxy-terminally truncated recombinant flavivirus envelope glycoproteins employing drosophila melanogaster expression systems

L5 ANSWER 13 OF 19 USPATFULL on STN
TI Bioluminescent bioreporter integrated circuit

L5 ANSWER 14 OF 19 USPATFULL on STN
TI Non-A, non-B, non-C, non-D, non-E hepatitis reagents and methods for their use

L5 ANSWER 15 OF 19 USPATFULL on STN
TI Method and system for enhanced production of commercially important exoproteins in gram-positive bacteria

L5 ANSWER 16 OF 19 USPATFULL on STN
TI Method and system for enhanced production of commercially important exoproteins in gram-positive bacteria

L5 ANSWER 17 OF 19 USPATFULL on STN
TI Expression vectors and methods for intracellular protein production in bascillus

L5 ANSWER 18 OF 19 USPATFULL on STN
TI Recombinant DNA-molecules and method for protein production

L5 ANSWER 19 OF 19 USPATFULL on STN
TI Method for the preparation of a selected protein or a part thereof in Bacillus strain bacteria

=> s l5 not l1
L6 18 L5 NOT L1

=> d l16,cbib,ab,clm,1-18
L16 NOT FOUND
The L-number entered has not been defined in this session, or it has been deleted. To see the L-numbers currently defined in this session, enter DISPLAY HISTORY at an arrow prompt (=>).

=> d l6,cbib,ab,clm,1-18

L6 ANSWER 1 OF 18 USPATFULL on STN
2005:283181 Chimeric flavivirus vaccines.
Chambers, Thomas J., St. Louis, MO, UNITED STATES
Monath, Thomas P., Harvard, MA, UNITED STATES
Guirakhoo, Farshad, Melrose, MA, UNITED STATES
Arroyo, Juan, S. Weymouth, MA, UNITED STATES
Acambis, Inc., Cambridge, MA, UNITED STATES (U.S. corporation)St. Louis University, St. Louis, MO, UNITED STATES (U.S. corporation)
US 6962708 B1 20051108

APPLICATION: US 1998-121587 19980723 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A chimeric live, infectious, attenuated virus containing a yellow fever virus, in which the nucleotide sequence for a prM-E protein is either deleted, truncated, or mutated, so that functional prM-E protein is not expressed, and integrated into the genome of the yellow fever virus, a nucleotide sequence encoding a prM-E protein of a second, different flavivirus, so that the prM-E protein of the second flavivirus is expressed.

CLM What is claimed is:

1. A chimeric live, infectious, attenuated virus, comprising: a yellow fever virus in which the nucleotide sequence encoding a prM-E protein is either deleted, truncated, or mutated so that functional yellow fever virus prM-E protein is not expressed, and integrated into the genome of said yellow fever virus, a nucleotide sequence encoding a prM-E protein of a second, different flavivirus, to that said prM-E protein of said second flavivirus is expressed, wherein the capsid protein of said chimeric virus is from yellow fever virus.

2. The chimeric virus of claim 1, wherein said second flavivirus is a Japanese Encephalitis (JE) virus.

3. The chimeric virus of claim 1, wherein the nucleotide sequence encoding the prM-E protein of said, second, different flavivirus replaces the nucleotide sequence encoding the prM-E protein of said yellow fever virus.

4. The chimeric virus of claim 1, wherein said nucleotide sequence encoding said prM-E protein of said second, different flavivirus comprises a mutation that prevents prM cleavage to produce M protein.

5. The chimeric virus of claim 1, wherein the NS2B-3-protease recognition site and the **signal** sequences and cleavage sites at the C/prM and E/NS1 junctions are maintained in construction of said chimeric virus.

6. The chimeric virus of claim 1, wherein said second flavivirus is a Murray Valley Encephalitis virus.

7. The chimeric virus of claim 1, wherein said second flavivirus is a St. Louis Encephalitis virus.

8. The chimeric virus of claim 1, wherein said second flavivirus is a West Nile virus.

9. The chimeric virus of claim 1, wherein said second flavivirus is a Tick-borne Encephalitis virus.

10. The chimeric virus of claim 1, wherein the **signal sequence** at the C/prM junction is maintained in construction of said chimeric virus.

11. A method of preventing or treating **Japanese encephalitis virus** infection in a patient, said method comprising administering to said patient a chimeric, live, infectious, attenuated virus comprising: a yellow fever virus in which the nucleotide sequence encoding a prM-E protein is either deleted, truncated, or mutated so that functional yellow fever virus prM-E protein is not expressed, and integrated into the genome of said yellow fever virus, a nucleotide sequence encoding a prM-E protein of **Japanese encephalitis virus** strain SA-14-14-2 or **Japanese encephalitis virus** strain Nakayama, wherein the capsid protein of said chimeric virus is from yellow fever virus.

12. The method of claim 11, wherein the nucleotide sequence encoding the prM-E protein of said **Japanese encephalitis virus** replaces the nucleotide sequence encoding the prM-E protein of said yellow fever virus.

13. The method of claim 11, wherein said nucleotide sequence encoding said prM-E protein of said **Japanese encephalitis virus** comprises a mutation that prevents prM cleavage to produce M protein.

14. The method of claim 11, wherein the NS2B-3 protease recognition site and the **signal** sequences and cleavage sites at the C/prM and E/NS1 junctions are maintained in connection of said chimeric virus.

L6 ANSWER 2 OF 18 USPATFULL on STN
2004:78909 Non-stochastic generation of genetic vaccines and enzymes.

Short, Jay M., Rancho Santa Fe, CA, United States
Diversa Corporation, San Diego, CA, United States (U.S. corporation)
US 6713279 B1 20040330
APPLICATION: US 2000-498557 20000204 (9)
PRIORITY: US 1995-8311P 19951207 (60)
US 1995-8316P 19951207 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by use of non-stochastic methods of directed evolution (DirectEvolution.TM.). These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). Through use of the claimed methods, genetic vaccines, enzymes, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.
CLM What is claimed is:
1. A method of providing an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, thereby providing an immunomodulatory polynucleotide.

2. The method of claim 1 wherein the library of non-stochastically generated progeny polynucleotides is optimized by directed evolution of the parental polynucleotides, such that polypeptides encoded by the optimized progeny polynucleotides are enhanced in their modulatory effect on an immune response.

3. The method of claim 2, wherein said progeny polynucleotide whose modulatory effect on an immune response is optimized by directed evolution is introduced into a genetic vaccine vector.

4. The method of claim 2, wherein said method of directed evolution is selected from the group consisting of codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, oligonucleotide-directed saturation mutagenesis, in vivo reassortment of polynucleotide sequences having partial homology, naturally occurring recombination processes which reduce sequence complexity, and any combination thereof.

5. The method of claim 4, wherein the method of directed evolution introduces at least at least one point mutation, addition, deletion, or chimerization, from one or more parental polynucleotides.

6. The method of claim 1, further comprising screening said library for progeny polynucleotides which encode polypeptides optimized for their immunomodulatory effect as compared to the parental polynucleotides.

7. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that interacts with a cellular receptor.

8. The method of claim 7, wherein the cellular receptor is a macrophage scavenger receptor.

9. The method of claim 7, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine receptor.

10. The method of claim 9, wherein the chemokine receptor is CCR6.

11. The method of claim 7, wherein the polypeptide acts as an agonist or antagonist of the receptor.

12. The method of claim 1, wherein the library is screened by contacting replicable genetic packages, which express the encoded polypeptides of the optimized progeny polynucleotides as fusions with proteins displayed on the surface, with a plurality of cells that display the receptor.

13. The method of claim 12, further comprising identifying cells that exhibit a modulation of an immune response by the receptor.

14. The method of claim 12, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.

15. The method of claim 14, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.

16. The method of claim 1, further comprising introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a subject.

17. The method of claim 16, wherein the peptide or polypeptide is an agonist or antagonist of the receptor.

18. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.

19. The method of claim 18, wherein the optimized non-stochastically generated polypeptide is introduced into a nucleotide sequence that encodes an HBsAg polypeptide.

20. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.

21. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that inhibits an allergic reaction.

22. The method of claim 21, wherein the polypeptide is selected from the group consisting of interferon-α, interferon-β, IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.

23. The method of claim 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.

24. The method of claim 23, wherein the antagonist of IL-10 is soluble or defective IL-10 receptor or IL-20/MDA-7.

25. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

26. The method of claim 25, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86).

27. The method of claim 26 wherein the screening step involves selecting variants with altered activity through CD28 or CTLA-4.

28. The method of claim 25, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).

29. The method of claim 25, wherein the co-stimulator is a cytokine.

30. The method of claim 29, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF-α, IFN-α, IFN-γ, and IL-20 (MDA-7).

31. The method of 30, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.

32. The method of claim 31, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.

33. The method of 30, wherein the cytokine is interleukin-12 and screening is performed by growing mammalian cells which contain the genetic vaccine vector in a culture medium and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.

34. The method of 30, wherein the cytokine is interferon-γ.

35. The method of claim 34, wherein the library is screened by contacting replicable genetic packages, which express the encoded polypeptides of the optimized progeny polynucleotides as fusions with proteins displayed on the surface, with a plurality of B cells that display the receptor.

36. The method of claim 35, further comprising identifying phage library members that are capable of inhibiting proliferation of the B cells.

37. The method of claim 30, wherein the immune response of interest is differentiation of T cells to T_{H1} cells.

38. The method of claim 37, wherein said immune response of interest is screened by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon-γ.

39. The method of claim 29, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide.

40. The method of claim 39, wherein the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a subject and determining whether an immune response is induced against the cytokine.

41. The method of claim 31, wherein the cell is tested for ability to costimulate an immune response.

42. The method of claim 1, wherein the optimized recombinant polynucleotide encodes a cytokine antagonist.

43. The method of claim 42, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor, a transmembrane cytokine receptor having a defective **signal sequence**, IL-10R and IL-4R.

44. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H1} immune response.

45. The method of claim 1, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H2} immune response.

46. The method of claim 1, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response.

47. The method of claim 46, wherein said method generates a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule.

48. The method of claim 47, wherein said recipient can be a human or an animal host.

49. The method of claim 48, wherein said method generates a molecule having decreased antigenicity with respect to at least one host recipient of said molecule.

50. The method of claim 49, wherein said recipient can be a human or an animal host.

51. The method of claim 1, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response and an increase in a second desirable modulatory effect on an immune response.

52. The method of claim 51, wherein the first and the second recipient hosts can be the same or different.

53. The method of claim 51, wherein each of the first and the second recipient hosts can be human or animal.

54. The method of claim 51, wherein said method generates a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule and an increased ability to elicit a second immune response from a second host recipient of said molecule.

55. The method of claim 54, wherein the first and the second recipient hosts can be the same or different.

56. The method of claim 54, wherein each of the first and the second recipient hosts can be a human or animal.

57. The method of claim 51, wherein said method generates a molecule having both a first decreased antigenicity with respect to at least one host recipient of said molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.

58. The method of claim 46, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector.

59. The method of claim 58, wherein said module is selected from the group of modules consisting of an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin of replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker, an enhancer, a promoter, an operator, an intron, or derivative fragments or analogs thereof, and any combination thereof.

60. The method of claim 1, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby.

61. The method of claim 60, wherein said method generates a molecule having an increased stability ex vivo.

62. The method of claim 60 wherein said method generates a molecule having increased stability in vivo, with respect to any means of biological elimination or degradation, upon administration to a host recipient.

63. The method of claim 1, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby has an optimized modulatory effect on an immune response in an animal or human host recipient.

64. The method of claim 63, wherein said method generates an optimized genetic vaccine for any human and/or non-human recipients.

65. A method of providing an optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response said method comprising non-stochastically reassembling at least two parental template polynucleotides, each of which encodes a molecule that is involved in modulating an immune response, thereby providing a library of non-stochastically generated polynucleotides.

66. The method of claim 65, wherein the first and second parental templates differ from each other in two or more nucleotides.

67. The method of claim 65, further comprising screening the library to identify at least one optimized non-stochastically generated polynucleotide that exhibits through the encoded molecule an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.

68. The method of claim 65, wherein an optimized non-stochastically generated polynucleotide is subjected to at least one further round of non-stochastic reassembly with at least one additional polynucleotide to produce additional working libraries of recombinant polynucleotides.

69. The method of claim 68, wherein said additional working libraries are screened to identify at least one further optimized non-stochastically generated polynucleotide which encodes a polypeptide that has been optimized for its immunomodulatory effect when compared to the parental polynucleotide from which the library was created.

70. A method of providing an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, said method comprising creating a library of

non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule.

71. The method of claim 70, further comprising screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell as compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly.

72. The method of claim 70, wherein said method of directed evolution is selected from the group consisting of codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, oligonucleotide-directed saturation mutagenesis, in vivo reassortment of polynucleotide sequences having partial homology, naturally occurring recombination processes which reduce sequence complexity, and any combination thereof.

73. The method of claim 70, wherein said method generates an optimized molecule for any human and/or non-human recipients.

74. The method of claim 70, further comprising forming a library of vectors by introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen.

75. The method of claim 74, wherein the library of vectors is introduced into mammalian cells.

76. The method of claim 75, wherein said cells that exhibit increased or decreased immunogenicity to the antigen are identified.

77. The method of claim 70, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.

78. The method of claim 70, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.

79. The method of claim 78, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.

80. The method of claim 70, wherein the accessory molecule comprises an immunogenic agonist sequence.

81. A method of providing an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, said method comprising creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, thereby providing an immunomodulatory polynucleotide.

82. The method of claim 81, wherein the library of non-stochastically generated polynucleotides is optimized by directed evolution.

83. The method of claim 82, wherein said method of directed evolution is selected from the group consisting of codon site-saturation mutagenesis, amino acid site-saturation mutagenesis, gene site saturation mutagenesis, introduction of mutations by non-stochastic polynucleotide reassembly methods, synthetic ligation polynucleotide reassembly, gene reassembly, oligonucleotide-directed saturation mutagenesis, in vivo reassortment of polynucleotide sequences having partial homology, naturally occurring recombination processes which reduce sequence complexity, and any combination thereof.

84. The method of claim 82, further comprising screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host as compared to the expression of parental polynucleotides.

85. The method of claim 82, wherein the recombinant expression host is a prokaryote.

86. The method of claim 82, wherein the recombinant expression host is a eukaryote.

87. The method of claim 86, wherein the recombinant expression host is a plant.

88. The method of claim 87, wherein the recombinant expression host is a monocot or dicot.

89. A method of producing a progeny polynucleotide set by subjecting a double-stranded circular parental polynucleotide molecule to mutagenesis, said method comprising synthesizing by means of a polymerase-catalyzed amplification reaction a first progeny polynucleotide strand comprised of said first primer and a second progeny polynucleotide strand comprised of said second primer, wherein the first progeny polynucleotide strand and the second progeny polynucleotide strand form a double-stranded mutagenized circular polynucleotide product, and wherein at least one of said primers contains a non-stochastic mutagenic cassette with respect to the parental polynucleotide molecule, thereby producing a progeny polynucleotide set.

90. The method of claim 89, wherein said non-stochastic mutagenic cassette contained in said at least one primer is degenerate in nature.

91. The method of claim 90, wherein a degenerate progeny polynucleotide set is produced.

92. A method of producing a set of progeny polypeptides, in which a non-stochastic range of single amino acid substitutions is represented at each amino acid position, from a template polypeptide set, said method comprising subjecting a codon-containing template polynucleotide to polymerase-based amplification using a degenerate oligonucleotide for each codon to be mutagenized.

93. The method of claim 92, wherein said method generates from at least one to twenty different amino acids at each amino acid site along a parental polypeptide template.

94. The method of claim 92, wherein said degenerate oligonucleotides is comprised of a first homologous sequence and a degenerate trinucleotide cassette.

95. The method of claim 92, wherein said degenerate oligonucleotide is comprised of a first homologous sequence, a degenerate trinucleotide cassette, and a second homologous sequence.

96. The method of claim 92, wherein said degenerate trinucleotide cassette is comprised of a first mononucleotide cassette selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette, a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, and a degenerate A/C/G/T mononucleotide cassette.

97. The method of claim 96, wherein said degenerate trinucleotide cassette is further comprises a second and a third mononucleotide cassette, each selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, a degenerate A/C/G/T mononucleotide cassette, a non-degenerate A mononucleotide cassette, and a non-degenerate C mononucleotide cassette, a non-degenerate G mononucleotide cassette, and a non-degenerate T mononucleotide cassette.

98. The method of claim 92, where said degenerate trinucleotide cassette is selected from the group consisting of: a degenerate N,N,N trinucleotide cassette, a degenerate N,N,G/T trinucleotide cassette, a degenerate N,N,G/C trinucleotide cassette, a degenerate N,N,A/C/G trinucleotide cassette, a degenerate N,N,A/G/T trinucleotide cassette, and a degenerate N,N,C/G/T trinucleotide cassette.

99. The method of claim 92, wherein said degenerate oligonucleotide is comprised of a first homologous sequence and a plurality of trinucleotide cassettes.

100. The method of claim 99, wherein said method generates a progeny polypeptide having a plurality of concurrent single amino acid changes, at each amino acid site, with respect to a parental polvpeptide template.

101. The method of claim 9, wherein each of said degenerate trinucleotide cassettes is comprised of a first mononucleotide cassette selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette, a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, and a degenerate A/C/G/T mononucleotide cassette.

102. The method of claim 101, wherein each of said degenerate trinucleotide cassettes further comprises a second and third mononucleotide cassette, each selected from the group consisting of: a degenerate A/C mononucleotide cassette, a degenerate A/G mononucleotide cassette, a degenerate A/T mononucleotide cassette, a degenerate C/G mononucleotide cassette, a degenerate C/T mononucleotide cassette, a degenerate G/T mononucleotide cassette, a degenerate C/G/T mononucleotide cassette a degenerate A/G/T mononucleotide cassette, a degenerate A/C/T mononucleotide cassette, a degenerate A/C/G mononucleotide cassette, a degenerate A/C/G/T mononucleotide cassette, a non-degenerate A mononucleotide cassette, a non-degenerate C mononucleotide cassette, a non-degenerate G mononucleotide cassette, and a non-degenerate T mononucleotide cassette.

103. The method of claim 99, where said degenerate trinucleotide cassette is selected from the group consisting of: a degenerate N,N,N trinucleotide cassette, a degenerate N,N,G/T trinucleotide cassette, a degenerate N,N,G/C trinucleotide cassette, a degenerate N,N,A/C/G trinucleotide cassette, a degenerate N,N,A/G/T trinucleotide cassette, and a degenerate N,N,C/G/T trinucleotide cassette.

104. The method of claim 92, wherein said degenerate oligonucleotide is comprised of a first homologous sequence, and a plurality of trinucleotide cassettes, and a second homologous sequence.

105. The method of claim 92, further comprising screening the progeny polypeptides to identify those that display a desirable change with respect to at least one molecular property as compared with its parental polypeptide.

L6 ANSWER 3 OF 18 USPATFULL on STN
2004:46740 Chimeric flavivirus vaccines.
Chambers, Thomas J., St. Louis, MO, United States
Monath, Thomas P., Harvard, MA, United States
Guirakhoo, Farshad, Melrose, MA, United States
Acambis, Inc., Cambridge, MA, United States (U.S. corporation)St. Louis University, St. Louis, MO, United States (U.S. corporation)
US 6696281 B1 20040224
APPLICATION: US 1999-452638 19991201 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A chimeric live, infectious, attenuated virus containing a yellow fever virus, in which the nucleotide sequence for a prM-E protein is either deleted, truncated, or mutated, so that functional prM-E protein is not expressed, and integrated into the genome of the yellow fever virus, a nucleotide sequence encoding a prM-E protein of a second, different flavivirus, so that the prM-E protein of the second flavivirus is expressed.
CLM What is claimed is:
1. A chimeric live, infectious, attenuated virus, comprising a yellow fever virus in which the nucleotide sequences encoding the pre-membrane and envelope proteins are replaced with the nucleotide sequences encoding pre-membrane and envelope proteins of a Dengue virus.

2. The chimeric virus of claim 1, wherein said Dengue virus is selected from the group consisting of Dengue types 1, 2, 3, and 4.

3. The chimeric virus of claim 2, wherein said nucleotide sequences derived from said Dengue virus are derived from two or more different Dengue strains.

4. The chimeric virus of claim 1, wherein said nucleotide sequences encoding said pre-membrane and envelope proteins of said Dengue virus comprise a substitution or deletion in the R-X-R/K-R sequence at the cleavage site of the pre-membrane protein that prevents cleavage of the pre-membrane protein to produce the membrane protein.

5. The chimeric virus of claim 1, wherein said chimeric virus comprises a **signal sequence** at the amino acid terminus of said pre-membrane protein, and said **signal sequence** is that of yellow fever virus.

6. The chimeric virus of claim 1, wherein said Dengue virus is a Dengue-1 virus.

7. The chimeric virus of claim 1, wherein said Dengue virus is a Dengue-2 virus.

8. The chimeric virus of claim 1, wherein said Dengue virus is a Dengue-3 virus.

9. The chimeric virus of claim 1, wherein said Dengue virus is a Dengue-4 virus.

L6 ANSWER 4 OF 18 USPATFULL on STN
2003:271094 Novel co-stimulatory molecules.
Punnonen, Juha, Belmont, CA, UNITED STATES
Lazetic, Alexandra L.L., San Jose, CA, UNITED STATES
Leong, Steven R., Berkeley, CA, UNITED STATES
Chang, Chia-Chun Jean, Los Gatos, CA, UNITED STATES
Apt, Doris, Sunnyvale, CA, UNITED STATES
Gustafsson, Claes, Belmont, CA, UNITED STATES
US 2003190697 A1 20031009
APPLICATION: US 2001-888324 A1 20010622 (9)
PRIORITY: US 2000-213946P 20000623 (60)
US 2000-241245P 20001017 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides polynucleotides and polypeptides encoded therefrom having advantageous properties, including an ability of the polypeptides to preferentially bind a CD28 or CTLA-4 receptor at a level greater or less than the ability of human B7-1 to bind CD28 or CTLA-4, or to induce or inhibit altered level of T cell proliferation response greater compared to that generated by human B7-1. The polypeptides and polynucleotides of the invention are useful in therapeutic and prophylactic treatment methods, gene therapy applications, and vaccines.
CLM What is claimed is:
1. An isolated or recombinant polypeptide comprising an extracellular domain sequence, wherein said extracellular domain sequence has at least about 75% amino acid sequence identity to an extracellular domain sequence of at least one of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293, and is not a naturally-occurring extracellular domain sequence, and wherein said polypeptide has a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

2. The isolated or recombinant polypeptide of claim 1, wherein said extracellular domain sequence has at least about 90% sequence identity to an extracellular domain sequence of at least one of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293.

3. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an extracellular domain sequence of any one of SEQ ID NOS: 48-68, 174-182, 184-221, 283-285, and 290-293.

4. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293.

5. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

6. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

7. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has a decreased or a lowered binding affinity for CTLA-4 as

compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

8. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide induces T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

9. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide induces T-cell proliferation.

10. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide induces a T-cell proliferative response equal to or greater than that of human B7-1.

11. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

12. The isolated or recombinant polypeptide of claim 5, which polypeptide comprises an extracellular domain sequence of any one of SEQ ID NOS: 48-68 and 174-209.

13. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an extracellular domain sequence encoded by a coding polynucleotide sequence, the coding polynucleotide sequence selected from the group of: (a) an extracellular domain coding sequence of a polynucleotide sequence selected from any of SEQ ID NOS: 1-21 and 95-142; (b) an polynucleotide sequence that encodes the extracellular domain of a polypeptide selected from any of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293; and (c) a polynucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a polynucleotide sequence (a) or (b).

14. An isolated or recombinant polypeptide, which polypeptide comprises a non-naturally-occurring amino acid sequence encoded by a nucleic acid comprising a polynucleotide sequence selected from the group of: (a) a polynucleotide sequence selected from SEQ ID NOS: 1-21 and 95-142, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); (d) a polynucleotide sequence comprising all or a fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide having a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1; (e) a polynucleotide sequence encoding a polypeptide, the polypeptide comprising an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293; and (f) a polynucleotide sequence encoding a polypeptide that has a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, which polynucleotide sequence has at least about 70% identity to at least one polynucleotide sequence of (a), (b), (c), or (d).

15. The isolated or recombinant polypeptide of claim 14, the polypeptide comprising an amino acid sequence of any one of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293.

16. The isolated or recombinant polypeptide of claim 14, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

17. The isolated or recombinant polypeptide of claim 14, wherein the polypeptide induces T-cell proliferation.

18. The isolated or recombinant polypeptide of claim 14, wherein the polypeptide induces a T-cell proliferative response equal to or greater than that of human B7-1.

19. An isolated or recombinant polypeptide comprising a sequence according to the formula: MGHTM-X6-W-X8-SLPPK-X14-PCL-X18-X19-X20-QLLVLT-X27-LFYFCSGITPKSVTKRVKETVMLSCDY-X55-TSTE-X60-LTSLRIYW-X69-KDSKMVLAILPGKVQVWPEYKNRTITDMNDN-X101-RIVI-X106-ALR-X110-SD-X113-GTYTCV-X120-QKP-X124-LKGAYKLEHL-X135-SVRLMIRADFPVP-X149-X150-X151-DLGNPSPNIRRLICS-X167-X168-X169-GFPRPHL-X177-WLENGEELNATNTT-X192-SQDP-X197-T-X199-LYMISSEL-X208-FNVTNN-X215-SI-X218-CLIKYGEL-X227-VSQIFPWSKPKQEPPIDQLPF-X249-VIIPVSGALVL-X261-A-X263-VLY-X267-X268-ACRH-

X273-ARWKRTRRNEETVGTE RLSPIYLGSAQSSG (SEQ ID NO: 284), or a subsequence thereof comprising the extracellular domain, wherein position X6 is Lys or Glu; position X8 is Arg or Gly; position X14 is Arg or Cys; position X18 is Trp or Arg; position X19 is Pro or Leu; position X20 is Ser or Pro; position X27 is Asp or Gly; position X55 is Asn or Ser; position X60 is Glu or Lys; position X69 is Gln or Arg; position X101 is Pro or Leu; position X106 is Leu or Gln; position X110 is Pro or Leu; position X113 is Lys or Ser; position X120 is Val or Ile; position X124 is Val or Asp; position X135 is Thr or Ala; position X149 is Thr, Ser, or del; position X150 is Ile or del; position X151 is Asn or Thr; position X167 is Thr or del; position X169 is Ser or del; position X169 is Gly or del; position X177 is Cys or Tyr; position X192 is Val or Leu; position X197 is Gly or Glu; position X199 is Glu or Lys; position X208 is Gly or Asp; position X215 is His or Arg; position X218 is Ala or Val; position X227 is Ser or Leu; position X249 is Trp, Leu, or Arg; position X261 is Ala or Thr; position X263 is Val, Ala, or Ile; position X267 is Arg or Cys; position X268 is Pro or Leu; and position X273 is Gly or Val.

20. The isolated or recombinant polypeptide of claim 19, which polypeptide comprises an extracellular domain sequence of any one of SEQ ID NOS: 51-56, 58, 61, 66, 67, 174-179, 181, 185-187, 189, 192-194, 197, 199, 202, 205, 208, 215, 217, 220, and 285.

21. The isolated or recombinant polypeptide of claim 19, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

22. The isolated or recombinant polypeptide of claim 19, wherein the polypeptide induces T-cell proliferation.

23. The isolated or recombinant polypeptide of claim 19, wherein the polypeptide induces a T-cell proliferative response equal to or greater than that of human B7-1.

24. The isolated or recombinant polypeptide of claim 19, comprising three or more of: Lys at position X6; Arg at position X8; Arg at position X14; Trp at position X18; Pro at position X19; Ser at position X20; Asp at position X27; Asn at position X55; Leu at position X106; Pro at position X110; Lys at position X113; Val at position X120; Val at position X124; Thr at position X135; Asn at position X151; Cys at position X177; Val at position X192; Gly at position X197; Glu at position X199; Gly at position X208; His at position X215; Ala at position X218; Trp at position X249; Ala at position X261; Val at position X263; Arg at position X267; Pro at position X268; and Gly at position X273.

25. The isolated or recombinant polypeptide of claim 24, comprising three or more of: Arg at position X8; Arg at position X14; Trp at position X18; Pro at position X19; Ser at position X20; Pro at position X110; Val at position X120; Val at position X124; Cys at position X177; Val at position X192; Gly at position X197; Glu at position X199; Gly at position X208; His at position X215; Ala at position X218; Trp at position X249; Ala at position X261; and Val at position X263.

26. The isolated or recombinant polypeptide of claim 25, comprising the extracellular domain sequence of SEQ ID NO: 66 or SEQ ID NO: 285.

27. The isolated or recombinant polypeptide of claim 25, comprising the sequence SEQ ID NO: 66 or SEQ ID NO: 285.

28. An isolated or recombinant polypeptide comprising a subsequence of an amino acid sequence set forth in any of SEQ ID NOS: 48-68, 174-182, 184-221, 283-285, and 290-293, wherein the subsequence is the extracellular domain of said amino acid sequence.

29. The isolated or recombinant polypeptide of claim 1, 14, 19, or 28, comprising a **signal sequence**.

30. The polypeptide of claim 1, 14, 19, or 28, wherein the **signal sequence** is selected from the **signal sequence** set forth in any of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293.

31. The polypeptide of claim 1, 14, 19, or 28, comprising a transmembrane domain sequence or a cytoplasmic domain sequence selected from the transmembrane domain sequence or the cytoplasmic domain sequence set forth in any of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293.

32. The polypeptide of claim 1, 14, 19, or 28 comprising a soluble

extracellular domain of a NCSM or a fragment or subsequence thereof.

33. The polypeptide of claim 1, 14, 19, or 28, wherein the polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.

34. The polypeptide of claim 33, wherein the at least one additional amino acid sequence comprises an Ig polypeptide.

35. The polypeptide of claim 34, wherein the Ig polypeptide is a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

36. The polypeptide of claim 1, 14, 19, or 28, comprising a polypeptide purification subsequence.

37. The polypeptide of claim 36, wherein the polypeptide purification subsequence is selected from: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

38. The polypeptide of claim 1, 14, 19, or 28, comprising a modified amino acid.

39. The polypeptide of claim 38, wherein the modified amino acid is selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

40. A composition comprising at least one polypeptide of claim 38 and a pharmaceutically acceptable excipient.

41. A composition comprising at least one polypeptide of claim 1, 14, 19, or 28, and a pharmaceutically acceptable excipient.

42. A composition comprising: an isolated or recombinant NCSM polypeptide comprising the amino acid sequence SEQ ID NOS: 48-68, 174-221, 283-285, 290-293, or a costimulatory fragment thereof, wherein said costimulatory fragment has a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, and a carrier.

43. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS: 1-21 and 95-142, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); and (d) a polynucleotide sequence comprising all or a fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide having a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

44. An isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding a polypeptide, wherein the encoded polypeptide comprises an amino acid sequence which is (a) substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293 and (b) is a non naturally-occurring sequence.

45. The nucleic acid of claim 44, wherein the encoded polypeptide is substantially identical over at least about 175 contiguous amino acid residues of any one of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293.

46. An isolated or recombinant nucleic acid comprising a nucleotide sequence coding for a polypeptide comprising the amino acid sequence set forth in any of SEQ ID NOS: 48-68, 174-221, 283-285, and 290-293, or a subsequence thereof, wherein the subsequence comprises at least one of: the **signal sequence** of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide, and wherein the amino acid sequence or subsequence is a non naturally-occurring sequence.

47. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

48. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has

either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

49. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

50. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide induces T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

51. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

52. The nucleic acid of claim 43, 44, or 46, wherein the nucleic acid encodes a fusion protein comprising at least one additional amino acid sequence.

53. The nucleic acid of claim 52, wherein the at least one additional amino acid sequence comprises an Ig polypeptide.

54. The nucleic acid of claim 53, wherein the Ig polypeptide is a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

55. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide comprises a **signal sequence**.

56. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide comprises a precursor peptide.

57. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide comprises an epitope tag sequence.

58. A cell comprising the nucleic acid of claim 43, 44, or 46.

59. The cell of claim 58, wherein the cell expresses a polypeptide encoded by the nucleic acid.

60. A vector comprising the nucleic acid of claim 43, 44, or 46.

61. The vector of claim 60, wherein the vector comprises a plasmid, a cosmid, a phage, a virus, or a fragment of a virus.

62. The vector of claim 60, wherein the vector is an expression vector.

63. The expression vector of claim 62, wherein the nucleic acid is operably linked to a promoter.

64. The expression vector of claim 62, further comprising a polynucleotide sequence encoding an antigen.

65. The expression vector of claim 64, wherein the antigen is a cancer antigen.

66. The expression vector of claim 64, wherein the nucleic acid is operably linked to first promoter and the polynucleotide sequence encoding the antigen is operably linked to a second promoter.

67. The expression vector of claim 65, wherein the cancer antigen is EpCam/KSA.

68. The expression vector of claim 67, wherein the expression vector comprises the vector shown in FIG. 22B.

69. A host cell comprising the vector of claim 60.

70. A composition comprising the nucleic acid of claim 43, 44, or 46 and an excipient.

71. The composition of claim 70, wherein the excipient is a pharmaceutically acceptable excipient.

72. A composition of matter comprising at least one nucleic acid of claim 43, 44, or 46.

73. The composition of claim 72, wherein the composition comprises a

library comprising at least about 2, 5, 10, 50 or more nucleic acids.

74. A composition produced by cleaving at least one nucleic acid of claim 43, 44, or 46.

75. The composition of claim 74, wherein the cleaving comprises mechanical, chemical, or enzymatic cleavage.

76. The composition of claim 75, wherein the enzymatic cleavage comprises cleavage with a restriction endonuclease, an RNAse, or a DNAse.

77. A composition produced by a process comprising incubating at least one nucleic acid of claim 43, 44, or 46 in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase.

78. The composition of claim 77, wherein the nucleic acid polymerase is a thermostable polymerase.

79. An isolated or recombinant nucleic acid encoding a polypeptide that has a CD28/CTLA-4 binding affinity ratio equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, produced by mutating or recombining at least one nucleic acid of claim 43, 44, or 46.

80. An isolated or recombinant polypeptide comprising a sequence having at least about 95% identity to at least about one of SEQ ID NOS: 69-92, 222-252, 286-289, or a subsequence thereof comprising the extracellular domain, wherein said sequence (a) is a non naturally-occurring sequence, and (b) comprises at least one of: Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; or Thr at position 279, wherein the position number corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO: 278), wherein said polypeptide has a CTLA-4/CD28BP binding affinity ratio equal to or greater than the CTLA-4/CD28BP binding affinity ratio of human B7-1.

81. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises a sequence having at least about 98% identity to at least one of SEQ ID NOS: 69-92, 222-252, 286-289, or a subsequence thereof comprising the extracellular domain, said sequence comprising at least one of: Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; and Thr at position 279, wherein the position number corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO: 278).

82. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises a sequence having at least about 98% identity to at least one of SEQ ID NOS: 69-92, 222-252, and 286-289, said sequence comprising at least one of: Gly at position 2; Gly at position 8; Cys at position 27; His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; Arg at position 219; Thr at position 250; Arg at position 266; Lys at position 275; and Ser at position 276, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO: 278).

83. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises a sequence having at least about 98% identity to the extracellular domain of at least one of SEQ ID NOS: 69-92, 222-252, and 286-289, said sequence comprising at least one of: His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; and Arg at position 219, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO: 278).

84. The isolated or recombinant polypeptide of claim 83, wherein said polypeptide comprises a sequence having at least about 98% identity to the extracellular domain of at least one of SEQ ID NOS: 69-92, 222-252, 286-289, said sequence comprising at least two of: His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; and Arg at position 219, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO: 278).

85. The isolated or recombinant polypeptide of claim 84, wherein said polypeptide comprises an extracellular domain of any one of SEQ ID NOS: 81, 85, 86, 88, 90, and 91.

86. The isolated or recombinant polypeptide of claim 80, which polypeptide comprises an extracellular domain of any one of SEQ ID NOS: 69-92, 222-252, and 286-289.

87. The isolated or recombinant polypeptide of claim 80, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS: 69-92, 222-252, and 286-289.

88. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has a CTLA-4/CD28BP binding affinity ratio greater than the CTLA-4/CD28BP binding affinity ratio of human B7-1.

89. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

90. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has a decreased or a lowered binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

91. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide inhibits T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

92. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide inhibits T-cell proliferation.

93. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide induces a T-cell response less than that of human B7-1.

94. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

95. The isolated or recombinant polypeptide of claim 84, which polypeptide comprises an extracellular domain sequence of any one of SEQ ID NOS: 69-92 and 222-247.

96. The isolated or recombinant polypeptide of claim 80, which polypeptide comprises an extracellular domain sequence encoded by a coding polynucleotide sequence, the coding polynucleotide sequence selected from the group: (a) an extracellular domain coding sequence of a polynucleotide sequence selected from any of SEQ ID NOS: 22-45 and 143-173; (b) a polynucteotide sequence that encodes the extracellular domain of a polypeptide selected from any of SEQ ID NOS: 69-92, 222-252, and 286-289; and (c) a polynucleotide sequence which hybridizes under stringent conditions o ver substantially the entire length of a polynucleotide sequence (a) or (b).

97. An isolated or recombinant polypeptide comprising a sequence that differs from a primate B7-1 sequence in at least one mutation selected from: Ser 12 Pro; Leu 25 Met; Gly 27 Cys; Ser 29 Pro; Lys 40 Arg; His

52 Leu; Tyr 65 His; Glu 122 Asp; Glu 129 Lys; Thr 135 Met; Thr 164 Ala; Ser 174 Phe; Glu 196 Gly; Ala 199 Thr; Thr 210 Ala; Lys 219 Arg; Thr 234 Pro; Asp 241 Asn; Val 254 Ala; Arg 275 Lys; Arg 276 Ser; or Arg 279 Thr; the mutation being indicated relative to human B7-1 with the amino acid sequence shown in SEQ ID NO: 278, wherein said sequence does not occur in nature, and wherein said polypeptide has a CTLA-4/CD28BP binding affinity ratio equal to or greater than the CTLA-4/CD28BP binding affinity ratio of human B7-1.

98. The isolated or recombinant polypeptide of claim 97 wherein said sequence differs from said primate B7-1 sequence in at least two of said mutations.

99. The isolated or recombinant polypeptide of claim 97 wherein said primate B7-1 is human B7-1 (SEQ ID NO: 278).

100. The isolated or recombinant polypeptide of claim 99, wherein said sequence differs from the human B7-1 sequence in at least two of said mutations.

101. An isolated or recombinant polypeptide comprising a sequence, said sequence having at least about 75% identity to at least one of SEQ ID NOS: 263-272, or a subsequence thereof comprising the extracellular domain, wherein said sequence is not a naturally-occurring sequence, and wherein said polypeptide has a CTLA-4/CD28BP binding affinity ratio equal to or greater than the CTLA-4/CD28BP binding affinity ratio of human B7-1.

102. An isolated or recombinant polypeptide, which polypeptide comprises a non naturally-occurring amino acid sequence encoded by a nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS: 22-45, 143-173, 253-262, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS: 69-92, 222-247, 263-272, 286-289, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); (d) a polynucleotide sequence comprising all or a fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide having a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1; (e) a polynucleotide sequence encoding a polypeptide, the polypeptide comprising an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS: 69-92, 222-247, 263-272, 286-289, and (f) a polynucleotide sequence encoding a polypeptide that has a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, which polynucleotide sequence has at least about 70% identity to at least one polynucleotide sequence of (a), (b), (c), or (d).

103. The isolated or recombinant polypeptide of claim 102, the polypeptide comprising an amino acid sequence of any one of SEQ ID NOS: 69-92, 222-247, 263-272, and 286-289.

104. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

105. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide inhibits T-cell proliferation.

106. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide induces a T-cell response less than that of human B7-1.

107. An isolated or recombinant polypeptide comprising a sequence according to the formula: MGHTRRQGTSP-X12-KCPYLKFFQLLV-X25-ACL-X29-HLCSGVIHVT-X40-EVKEVATLSCGLNVSVEELAQTRIHWQKEKKMVLTM MSGDMNIWPEYKNRTIFDTNNLSIVILALRPSDEGTYECVVLKY-X122-KDAFKR-X129-HLAEVMLSVKAD FPTPSITDFEIPPSNIRRIICS -X164-SGGFPEPHLFWLENGEELNAINTTVSQDPE T-X196-LYTVSSKLDFNM TANHSFMCLI-X219-YGHLRVNQTFNWNTPKQEHFP-X241-NLLPSWA ITLSANGIFVICCLTYRFAPRCRERKSNETLRRESVCPV (SEQ ID NO: 287), or a subsequence thereof comprising the extracellular domain, wherein position X12 is Ser or Pro; position X25 is Leu or Met; position X29 is Ser or Pro; position X40 is Lys or Arg; position X122 is Glu or Asp; position X129 is Glu or Lys; position X164 is Thr or Ala; position X196 is Glu or Gly; position X219 is Lys or Arg; and position X241 is Asp or Asn.

108. The isolated or recombinant polypeptide of claim 107, which polypeptide comprises the extracellular domain of SEQ ID NO: 288 or SEQ ID NO: 289.

109. The isolated or recombinant polypeptide of claim 107, comprising the sequence SEQ ID NO: 288 or SEQ ID NO: 289.

110. The isolated or recombinant polypeptide of claim 107, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

111. The isolated or recombinant polypeptide of claim 107, wherein the polypeptide inhibits T-cell proliferation.

112. The isolated or recombinant polypeptide of claim 107, wherein the polypeptide induces a T-cell response less than that of human B7-1.

113. An isolated or recombinant polypeptide comprising a subsequence of an amino acid sequence set forth in any of SEQ ID NOS: 69-92, 222-247, 263-272, and 286-289, wherein the subsequence is the extracellular domain of said amino acid sequence.

114. The isolated or recombinant polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a **signal sequence**.

115. The polypeptide of claim 114, wherein the **signal sequence** is selected from the **signal sequence** set forth in any of SEQ ID NOS: 69-92, 222-247, 263-272, and 286-289.

116. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a transmembrane domain sequence or a cytoplasmic domain sequence, selected from the transmembrane domain sequence or the cytoplasmic domain sequence set forth in any of SEQ ID NOS: 69-92, 222-247, 263-272, and 286-289.

117. The polypeptide of claim 80, 97, 101, 102, 107, or 113 comprising a soluble extracellular domain of a NCSM or a fragment or subsequence thereof.

118. The polypeptide of claim 80, 97, 101, 102, 107, or 113, wherein the polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.

119. The polypeptide of claim 118, wherein the at least one additional amino acid sequence comprises an Ig polypeptide.

120. The polypeptide of claim 119, wherein the Ig polypeptide is a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

121. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a polypeptide purification subsequence.

122. The polypeptide of claim 121, wherein the polypeptide purification subsequence is selected from: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

123. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a modified amino acid.

124. The polypeptide of claim 123, wherein the modified amino acid is selected from the group consisting of: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

125. A composition comprising at least one polypeptide of claim 124 and a pharmaceutically acceptable excipient.

126. A composition comprising at least one polypeptide of claim 80, 97, 101, 102, 107, or 113, and a pharmaceutically acceptable excipient.

127. A composition comprising: an isolated or recombinant NCSM polypeptide comprising the amino acid sequence of SEQ ID NOS: 69-92, 222-247, 263-272, 286-289, or a costimulatory fragment thereof, wherein said costimulatory fragment has a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, and a carrier.

128. An isolated or recombinant nucleic acid comprising a polynucleotide

sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS: 22-45, 143-173, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS: 69-92, 222-247, 286-289, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); and (d) a polynucleotide sequence comprising all or a fragment of (a), (b), or (c); wherein (c) or (d) encodes a polypeptide having a non naturally-occurring sequence comprising at least one of: Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; and Thr at position 279, wherein the position number corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO: 278), and wherein said polypeptide has a CTLA-4/CD28BP binding affinity ratio equal to or greater than the CTLA-4/CD28BP binding affinity ratio of human B7-1.

129. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS: 253-262, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS: 263-272, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b) and encodes a polypeptide having a non naturally-occurring sequence; and (d) a polynucleotide sequence comprising all or a fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide having (i) a non naturally-occurring sequence and (ii) a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

130. An isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding a polypeptide, the encoded polypeptide comprising an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS: 69-92, 222-247, 263-272, and 286-289.

131. The nucleic acid of claim 44, wherein the encoded polypeptide is substantially identical over at least about 200 contiguous amino acid residues of any one SEQ ID NOS: 69-92, 222-247, 263-272, and 286-289.

132. An isolated or recombinant nucleic acid comprising a nucleotide sequence coding for a polypeptide comprising the amino acid sequence set forth in any of SEQ ID NOS: 69-92, 222-247, 263-272, and 286-289, or a subsequence thereof, wherein the subsequence comprises at least one of: the **signal sequence** of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide, and wherein the amino acid sequence or subsequence is a non naturally-occurring sequence.

133. The nucleic acid of claim 128, 129, 130, or 132, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

134. The nucleic acid of claim 128, 129, 130, or 132, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

135. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

136. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide inhibits T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

137. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

138. The nucleic acid of claim 128, 129, 130, or 132, wherein the nucleic acid encodes a fusion protein comprising at least one additional amino acid sequence.

139. The nucleic acid of claim 138, wherein the at least one additional amino acid sequence comprises an Ig polypeptide.

140. The nucleic acid of claim 139, wherein the Ig polypeptide is a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

141. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises a **signal sequence**.

142. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises a precursor peptide.

143. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises an epitope tag sequence.

144. A cell comprising the nucleic acid of claim 128, 129, 130, or 132.

145. The cell of claim 144, wherein the cell expresses a polypeptide encoded by the nucleic acid.

146. A vector comprising the nucleic acid of claim 128, 129, 130, or 132.

147. The vector of claim 146, wherein the vector comprises a plasmid, a cosmid, a phage, a virus, or a fragment of a virus.

148. The vector of claim 146, wherein the vector is an expression vector.

149. The expression vector of claim 148, wherein the nucleic acid is operably linked to a promoter.

150. The expression vector of claim 149, further comprising a polynucleotide sequence encoding an Ig polypeptide or fragment thereof.

151. The expression vector of claim 150, wherein the Ig polypeptide is a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

152. The expression vector of claim 150, wherein the promoter is a CMV promoter.

153. The expression vector of claim 150, further comprising a BGH polyA sequence.

154. A host cell comprising the vector of claim 146.

155. A composition comprising the nucleic acid of claim 128, 129, 130, or 132, and an excipient.

156. The composition of claim 155, wherein the excipient is a pharmaceutically acceptable excipient.

157. A composition of matter comprising at least one nucleic acid of claim 128, 129, 130, or 132.

158. The composition of claim 157, wherein the composition comprises a library comprising at least about 2, 5, 10, 50 or more nucleic acids.

159. A composition produced by cleaving at least one nucleic acid of claim 128, 129, 130, or 132.

160. The composition of claim 159, wherein the cleaving comprises mechanical, chemical, or enzymatic cleavage.

161. The composition of claim 160, wherein the enzymatic cleavage comprises cleavage with a restriction endonuclease, an RNAse, or a DNAse.

162. A composition produced by a process comprising incubating at least

one nucleic acid of claim 128, 129, 130, or 132 in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase.

163. The composition of claim 162, wherein the nucleic acid polymerase is a thermostable polymerase.

164. An isolated or recombinant nucleic acid encoding a polypeptide that has a CTLA-4/CD28 binding affinity ratio equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, produced by mutating or recombining at least one nucleic acid of claim 128, 129, 130, or 132.

165. An isolated or recombinant polypeptide comprising a sequence according to the formula: MGHTMKWGSLPPKRPCLWLSQLLVLTGLFYFCSGITPK SVTKRVKETVM-X50-SCDY-X55-X56-STEELTSLRIYWQKDSKMVL AILPGKVQVWPEYKNRTITD MNDNPRIVILALRLSD-X113-GTYTCV-X120-QK-X123-X124-X125-X126-G-X128-X129-X130-X131-EHL-X135-SV-X138-L-X140-IRADFPVPSITDIGHPAPNVK RIRCSASG-X170-FPEPRLAWMEDGEEL NAVNTTV-X193-X194-X195-LDTELYSVSSELD-X209-N-X211-TNNHSIVCLIKYGELSVSQIFPWSKPK QEPPIDQLPFWVI-X252-X253-VSGALVLTAVVLYCLACRHVAR (SEQ ID NO: 290), or a subsequence thereof comprising the extracellular domain, wherein position X50 is Leu or Pro; position X55 is Asn or Ser; position X56 is Ala or Thr; position X113 is Ser or Lys; position X120 is Ile or Val; position X123 is Pro or deleted; position X124 is Val, Asn, or Asp; position X125 is Leu or Glu; position X126 is Lys or Asn; position X128 is Ala or Ser; position X129 is Tyr or Phe; position X130 is Lys or Arg; position X131 is Leu or Arg; position X135 is Ala or Thr; position X138 is Arg or Thr; position X140 is Met or Ser; position X170 is Asp or Gly; position X193 is Asp or is deleted; position X194 is Gln or is deleted; position X195 is Asp or is deleted; position X211 is Val or Ala; position X252 is Ile or Val; and position X253 is Leu or Pro.

166. The isolated or recombinant polypeptide of claim 165, which polypeptide comprises a sequence of any one of SEQ ID NOS: 59, 62, 180, 184, 188, 195, 196, 200, 201, 204, 211, 213, 219, and 291.

167. An isolated or recombinant polypeptide comprising a sequence according to the formula: MGHTMKWG-X9-LPPKRPCLWLSQLLVLTGLFYFCSG-X35-TPKSVTKRV KETVMLSCDY-X55-TSTEELTSLRIYWQKDSKMVLAILPGKVQVW PEYKNRTITDMNDNPRIVILALR-X110-SDSGTYTCVIQKP-X124-LKGAYKLEBL-X135-SVRLMIRADFPVPTINLGNPSPNRRLICSTSGGFPRPHLYWLENG-X183-ELNATNTT-X192-SQDPETKLYMISSELDFN-X211-TSN-X215-X216-X217-LCLVKYGDLTVSQ-X231-FYWQESKPTPSANQHLTWTIIIPVSAFGISVIIAVI LTCLTCRNAAIRRQRRENEV-X288-M-X290-SCSQSP (SEQ ID NO: 292), or a subsequence thereof comprising the extracellular domain, wherein position X9 is Thr or Ser; position X35 is Ile or Thr; position X55 is Asn or Ser; position X110 is Leu or Pro; position X124 is Asp or Val; position X135 is Thr or Ala; position X183 is Lys or Glu; position X192 is Leu or Val; position X211 is Met or Thr; position X215 is His or is deleted; position X216 is Ser or is deleted; position X217 is Phe or is deleted; position X231 is Thr or Ser; position X288 is Lys or Glu; position X290 is Glu or Gln, and wherein said sequence is a non naturally-occurring sequence.

168. The isolated or recombinant polypeptide of claim 167, which polypeptide comprises a sequence of any one of SEQ ID NOS: 48, 182, 183, 212, 214, 216, 218, 221, and 293.

169. An isolated or recombinant polypeptide comprising the sequence SEQ ID NO: 93, SEQ ID NO: 94, or a subsequence thereof, wherein the subsequence comprises at least one of: the **signal sequence** of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide.

170. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NO: 46, SEQ ID NO: 47, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 93, SEQ ID NO: 94, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence encoding a subsequence of a polypeptide selected from SEQ ID NO: 93, SEQ ID NO: 94, or a complementary polynucleotide sequence thereof, wherein the subsequence comprises at least one of: the **signal sequence** of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide.

171. A polypeptide which is specifically bound by a polyclonal antisera raised against one or more antigen, the antigen comprising the sequence SEQ ID NOS: 48-94, 174-252, 263-272, 283-293, or a fragment thereof, wherein the antisera is subtracted with a polypeptide encoded by one or

more of GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

172. An antibody or antisera produced by administering the polypeptide of claim 1, 80, 101, or 169 to a mammal, which antibody specifically binds one or more antigen, the antigen comprising a polypeptide comprising one or more of the amino acid sequences SEQ ID NOS: 48-94, 174-252, 263-272, and 283-293, which antibody does not specifically bind to a polypeptide encoded by one or more of GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

173. An antibody or antisera which specifically binds a polypeptide, the polypeptide comprising a sequence selected from: SEQ ID NOS: 48-94, 174-252, 263-272, and 283-293, wherein the antibody does not specifically bind to a polypeptide encoded by one or more of GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

174. A method of producing a polypeptide, the method comprising: (a) introducing into a population of cells a nucleic acid of claim 43, 46, 128, 129, 132, or 170, the nucleic acid operatively linked to a regulatory sequence effective to produce the encoded polypeptide; (b) culturing the cells in a culture medium to produce the polypeptide; and (c) isolating the polypeptide from the cells or from the culture medium.

175. A method of producing a polypeptide, the method comprising (a) introducing into a population of cells a recombinant expression vector comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170; (b) culturing the cells in a culture medium to produce the polypeptide encoded by the expression vector; and (c) isolating the polypeptide from the cells or from the culture medium.

176. A method of producing a polypeptide, the method comprising: (a) introducing into a population of cells a recombinant expression vector comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170; (b) administering the expression vector into a mammal; and (c) isolating the polypeptide from the mammal or from a byproduct of the mammal.

177. A method of inducing T-cell proliferation, the method comprising: contacting the T-cell population with a polypeptide of claim 1, 80, 101, or 169, thereby inducing proliferation of the T-cells.

178. A method of inhibiting T-cell proliferation, the method comprising: contacting the T-cell population with a polypeptide of claim 1, 80, 101, or 169, thereby inhibiting proliferation of the T-cells.

179. A method of modifying T-cell proliferation, the method comprising: contacting the T-cell population with a polypeptide of claim 1, 80, 101, or 169, thereby modifying proliferation of the T-cells.

180. A method of modifying T-cell activation, the method comprising: contacting the T-cell population with a polypeptide of claim 1, 80, 101, or 169, thereby modifying activation of the T-cells.

181. The method of claim 177, 178, 179, or 180 wherein the T-cells are in culture.

182. A method of treating an autoimmune disorder or medical condition in a patient, the method comprising: administering to the patient an effective amount of the polypeptide of claim 1, 80, 101, or 169.

183. A method of treating an autoimmune disorder or medical condition in a patient, the method comprising: administering to the patient an appropriate amount of an expression vector comprising the nucleic acid of claim 1, 80, 101, or 169.

184. The method of claim 182, wherein the autoimmune disorder is selected from the group comprising: multiple sclerosis, rheumatoid arthritis, lupus erythematosus, psoriasis, and type I diabetes.

185. The method of claim 182, wherein the medical condition comprises allogeneic or xenogeneic grafts or transplants.

186. A method of treating a medical disorder in a patient, the method comprising: administering to the patient an effective amount of the polypeptide of claim 1, 80, 101, or 169.

187. The method of claim 186, wherein the medical condition comprises: cancer, viral infection (e.g. HIV), or bacterial infection.

188. In a method of treating a disorder treatable by administration of a co-stimulatory molecule to a subject, an improved method comprising: administering to the subject an effective amount of the polypeptide of claim 1, 80, 101, or 169.

189. The method of claim 188, wherein the disorder treatable by administration of a co-stimulatory molecule is selected from the group comprising: sclerosis, rheumatoid arthritis, lupus erythematosus, psoriasis, type I diabetes, allogeneic grafts, xenogeneic grafts, cancer, viral infection, and bacterial infection.

190. A method of recombination, the method comprising recursively recombining one or more nucleic acid of claim 43, 46, 128, 129, 132, or 170, with one or more additional nucleic acid.

191. The method of claim 190, wherein the additional nucleic acid encodes a co-stimulatory homologue or subsequence thereof.

192. The method of claim 190, wherein the recursive recombination produces at least one library of recombinant co-stimulatory homologue nucleic acids.

193. A nucleic acid library produced by the method of claim 192.

194. A population of cells comprising the library of claim 193.

195. A recombinant co-stimulatory homologue nucleic acid produced by the method of claim 191.

196. A cell comprising the nucleic acid of claim 195.

197. The method of claim 190, wherein the recursive recombination is performed in vitro.

198. The method of claim 190, wherein the recursive recombination is performed in vivo.

199. A method of producing a modified co-stimulatory nucleic acid homologue comprising mutating a nucleic acid of claim 43, 46, 128, 129, 132, or 170.

200. The modified co-stimulatory homologue nucleic acid homologue produced by the method of claim 199.

201. A computer or computer readable medium comprising a database comprising a sequence record comprising one or more character string corresponding to a nucleic acid or protein sequence selected from SEQ ID NOS: 1-272 and 283-293.

202. An integrated system comprising a computer or computer readable medium comprising a database comprising one or more sequence records, each comprising one or more character strings corresponding to a nucleic acid or protein sequence selected from SEQ ID NOS: 1-272 and 283-293, the integrated system further comprising a user input interface allowing a user to selectively view one or more sequence record.

203. The integrated system of claim 202, the computer or computer readable medium comprising an alignment instruction set which aligns the character strings with one or more additional character string

corresponding to a nucleic acid or protein sequence.

204. The integrated system of claim 203, wherein the instruction set comprises one or more of: a local homology comparison determination, a homology alignment determination, a search for similarity determination, and a BLAST determination.

205. The integrated system of claim 203, further comprising a user readable output element which displays an alignment produced by the alignment instruction set.

206. The integrated system of claim 202, the computer or computer readable medium further comprising an instruction set which translates one or more nucleic acid sequence comprising a sequence selected from SEQ ID NOS: 1-47, 95-173, and 253-262 into an amino acid sequence.

207. The integrated system of claim 202, the computer or computer readable medium further comprising an instruction set for reverse-translating one or more amino acid sequence comprising a sequence selected from SEQ ID NOS: 48-94, 174-252, 263-272, and 283-293, into a nucleic acid sequence.

208. The integrated system of claim 207, wherein the instruction set selects the nucleic acid sequence by applying a codon usage instruction set or an instruction set which determines sequence identity to a test nucleic acid sequence.

209. A method of using a computer system to present information pertaining to at least one of a plurality of sequence records stored in a database, said sequence records each comprising one or more character string corresponding to SEQ ID NOS: 1-272 and 283-293, the method comprising: (a) determining a list of one or more character strings corresponding to one or more of SEQ ID NOS: 1-272 and 283-293, or a subsequence thereof; (b) determining which character strings of said list are selected by a user; and (c) displaying the selected character strings, or aligning the selected character strings with an additional character string.

210. The method of claim 209, further comprising displaying an alignment of the selected character string with the additional character string.

211. The method of claim 209, further comprising displaying the list.

212. A nucleic acid which comprises a unique subsequence in a nucleic acid selected from SEQ ID NOS: 1-47, 95-173, and 253-262, wherein the unique subsequence is unique as compared to a nucleic acid corresponding to any of GenBank Nucleotide Accession No.: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

213. A polypeptide which comprises a unique subsequence in a polypeptide selected from: SEQ ID NOS: 48-94, 174-252, 263-272, and 283-293, wherein the unique subsequence is unique as compared to a polypeptide encoded by any of GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

214. A target nucleic acid which hybridizes under stringent conditions to a unique coding oligonucleotide which encodes a unique subsequence in a polypeptide selected from: SEQ ID NOS: 48-94, 174-252, 263-272, and 283-293, wherein the unique subsequence is unique as compared to a polypeptide encoded by any of GenBank Nucleotide Accession No.: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823,

and Y09950.

215. The nucleic acid of claim 214, wherein the stringent conditions are selected such that a perfectly complementary oligonucleotide to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5× higher **signal** to noise ratio than for hybridization of the perfectly complementary oligonucleotide to a control nucleic acid corresponding to any of GenBank Nucleotide Accession No.: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950, wherein the target nucleic acid hybridizes to the unique coding oligonucleotide with at least about a 2×higher **signal** to noise ratio as compared to hybridization of the control nucleic acid to the coding oligonucleotide.

216. A method of therapeutic or prophylactic treatment of a disease or disorder in a subject in need of such treatment, comprising: administering to the subject a polypeptide of claim 1, 80, 101, or 169 and an immunogen specific for said disease or disorder, wherein the combined amount of polypeptide and immunogen is effective to prophylactically or therapeutically treat said disease or disorder.

217. The method of claim 216, wherein the polypeptide is present in an amount sufficient to enhance, diminish or modify an immune response induced by the immunogen.

218. The method of claim 216, wherein a composition comprising the polypeptide, the immunogen, and a pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat said disease or disorder.

219. The method of claim 216, wherein the subject is a mammal.

220. The method of claim 219, wherein the mammal is a human.

221. The method of claim 216, wherein the polypeptide is administered in vivo to the subject.

222. The method of claim 216, wherein the polypeptide is administered in vitro or ex vivo to one or more cells of the subject.

223. A method of enhancing, diminishing, modifying, or potentiating an immune response in a subject, comprising: directly administering to the subject a polynucleotide comprising a nucleic acid sequence of claim 43, 46, 128, 129, 132, or 170, operably linked to a promoter sequence that controls the expression of said nucleic acid sequence, said polynucleotide being present in an amount sufficient that uptake of said polynucleotide into one or more cells of the subject occurs and sufficient expression of said nucleic acid sequence results to produce an amount of a polypeptide effective to enhance, diminish, or modify an immune response.

224. The method of claim 223, further comprising administering to the subject an antigen specific for the disease or disorder, wherein the polynucleotide is administered to the subject in an amount sufficient to enhance, diminish, or modify the immune response induced in the subject by the antigen.

225. The method of claim 223, wherein the polynucleotide further comprises a nucleotide sequence encoding for an antigen.

226. The method of claim 223, wherein the polynucleotide further comprises at least one additional nucleotide sequence encoding a cytokine, adjuvant, co-stimulatory molecule, or at least one additional nucleotide sequence comprising a promoter.

227. The method of claim 223, wherein the subject is a mammal.

228. The method of claim 227, wherein the mammal is a human.

229. The method of claim 223, wherein said polynucleotide comprises a vector.

230. A method of treating a disease or disorder in a subject in need of

such treatment, comprising: administering to the subject a polypeptide of claim 1, 80, 101, or 169 in an amount effective to treat said disease or disorder.

231. A method of therapeutic or prophylactic treatment of a disease or disorder in a subject in need of such treatment, comprising: administering to the subject a polypeptide of claim 32 or 117 and an immunogen specific for said disease or disorder, wherein the combined amount of polypeptide and immunogen is effective to prophylactically or therapeutically treat said disease or disorder.

232. The method of claim 231, wherein the polypeptide is present in an amount sufficient to enhance, diminish or modify an immune response induced by the immunogen.

233. The method of claim 231, wherein a composition comprising the polypeptide, the immunogen, and a pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat the disease or disorder.

234. The method of claim 231, wherein the subject is a mammal.

235. The method of claim 234, wherein the mammal is a human.

236. The method of claim 231, wherein the polypeptide is administered in vivo to the subject.

237. The method of claim 231, wherein the polypeptide is administered in vitro or ex vivo to one or more cells of the subject.

238. A method of treating a disease or disorder in a subject in need of such treatment, comprising: administering to the subject a polypeptide of claim 58 in an amount effective to treat the disease or disorder.

239. The isolated or recombinant polypeptide of claim 165, comprising three or more of: Leu at position X50; Asn at position X55; Ala at position X56; Ser at position X113; Ile at position X120; Pro at position X123; Val at position X124; Leu at position X125; Lys at position X126; Ala at position X128; Tyr at position X129; Lys at position X130; Leu at position X131; Ala at position X135; Arg at position X138; Met at position X140; Asp at position X170; Asp at position X193; Asp at position X194; Asp at position X195; Val at position X211; Ile at position X252; and Leu at position X253.

240. The isolated or recombinant polypeptide of claim 167, comprising three or more of: Thr at position X9; Ile at position X35; Asn at position X55; Leu at position X110; Asp at position X124; Thr at position X135; Lys at position X183; Leu at position X192; Met at position X211; His at position X215; Ser at position X216; Phe at position X217; Thr at position X231; Lys at position X288; and Glu at position X290.

241. A method of modulating or altering a T-cell response specific to an antigen in a subject, the method comprising administering to the subject at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS: 48-94, 174-252, 263-272 and 283-293 or fragment thereof, and a polynucleotide sequence encoding the antigen or antigenic fragment thereof, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to modulate or alter a T cell response.

242. The vector of claim 241, wherein the at least one polynucleotide sequence encoding a polypeptide comprises a polynucleotide sequence selected from any of SEQ ID NOS: 1-47, 95-173, and 253-262.

243. The method of claim 241, wherein the polypeptide or fragment thereof interacts with or binds a T cell surface receptor.

244. The method of claim 241, wherein the T-cell response is enhanced.

245. The method of claim 244, wherein the enhanced T cell response is sufficient to eliminate cells bearing the antigen or antigenic fragment thereof.

246. The method of claim 241, wherein the T-cell response is suppressed or inhibited.

247. The method of claim 241, wherein the antigen or antigenic fragment thereof is an antigen or antigenic fragment thereof of an infectious

agent or a cancer.

248. The method of claim 244, wherein the polypeptide comprises SEQ ID NO: 66 or the extracellular domain amino acid sequence thereof.

249. The method of claim 245, wherein the polypeptide comprises SEQ ID NO:86 or the extracellular domain amino acid sequence thereof.

250. The method of claim 244, wherein the at least one polynucleotide sequence encoding a NCSM polypeptide or fragment thereof is operably linked to a promoter in a first vector.

251. The method of claim 250, wherein the at least one polynucleotide sequence encoding the antigen or antigenic fragment thereof is operably linked to a promoter in the first vector.

252. The method of claim 250, wherein the at least one polynucleotide sequence encoding the antigen or antigenic fragment thereof is operably linked to a promoter in the a second vector.

253. A vector comprising at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS: 48-94, 174-252, 263-272 and 283-293 or fragment thereof, and a polynucleotide sequence encoding the antigen or antigenic fragment thereof, wherein the NCSM polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, and wherein each of the at least one polynucleotide sequences is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

254. The vector of claim 253, wherein the at least one polynucleotide sequence encoding a polypeptide comprises a polynucleotide sequence of any of SEQ ID NOS: 1-47, 95-173, and 253-262.

255. The vector of claim 253, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to enhance a T cell response such that cells expressing the antigen or antigenic fragment thereof are eliminated.

256. The vector of claim 253, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to inhibit a T cell response.

257. A vector comprising at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS: 48-94, 174-252, 263-272 and 283-293 or fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, wherein the at least one polynucleotide sequence is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

258. A method of modulating or altering an immune response in a subject, the method comprising introducing into cells of a tumor of the subject at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS: 48-94, 174-252, 263-272 and 283-293 or fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, and wherein the at least one polynucleotide sequence is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

L6 ANSWER 5 OF 18 USPATFULL on STN
2003:200896 Novel co-stimulatory molecules.
Punnonen, Juha, Belmont, CA, UNITED STATES
Lazetic, Alexandra, San Jose, CA, UNITED STATES
Leong, Steven R., Berkeley, CA, UNITED STATES
Chang, Chia-Chun, Los Gatos, CA, UNITED STATES
Apt, Doris, Sunnyvale, CA, UNITED STATES
Gustafsson, Claes, Belmont, CA, UNITED STATES
Maxygen, Inc., Redwood City, CA, UNITED STATES, 94063 (U.S. corporation)
US 2003138881 A1 20030724
APPLICATION: US 2001-32214 A1 20011220 (10)
PRIORITY: US 2000-213946P 20000623 (60)
US 2000-241245P 20001017 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides polynucleotides and polypeptides encoded

therefrom having advantageous properties, including an ability of the polypeptides to preferentially bind a CD28 or CTLA-4 receptor at a level greater or less than the ability of human B7-1 to bind CD28 or CTLA-4, or to induce or inhibit altered level of T cell proliferation response greater compared to that generated by human B7-1. The polypeptides and polynucleotides of the invention are useful in therapeutic and prophylactic treatment methods, gene therapy applications, and vaccines.

CLM What is claimed is:

1. An isolated or recombinant polypeptide comprising an amino acid sequence of an extracellular domain, wherein said extracellular domain amino acid sequence has at least about 75% amino acid sequence identity to an extracellular domain amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, and is not a naturally-occurring extracellular domain amino acid sequence, and wherein said polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

2. The isolated or recombinant polypeptide of claim 1, wherein said extracellular domain (ECD) amino acid sequence has at least about 90% sequence identity to an ECD amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

3. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an ECD amino acid sequence of any one of SEQ ID NOS:48-68, 174-182, 184-221, 283-285, and 290-293.

4. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

5. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

6. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

7. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

8. The isolated or recombinant polypeptide of claim 1, 5, 6, or 7, wherein the polypeptide has an ability to induce T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation and further comprises at least transmembrane domain (TMD) and/or a cytoplasmic, wherein said transmembrane domain is not a naturally-occurring TMD.

9. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide induces T-cell proliferation.

10. The isolated or recombinant polypeptide of claim 1, 5, 6, or 7, wherein the polypeptide has an ability to induce a T-cell proliferative response about equal to or greater than that of human B7-1.

11. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has an ability to modulate T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

12. The isolated or recombinant polypeptide of claim 5, which polypeptide comprises an extracellular domain amino acid sequence of any one of SEQ ID NOS:48-68 and 174-209.

13. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an extracellular domain (ECD) amino acid sequence encoded by an ECD coding nucleotide sequence, the ECD coding nucleotide sequence selected from the group of: (a) a nucleotide sequence comprising a nucleotide fragment of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide fragment encodes an ECD amino acid sequence; (b) a nucleotide sequence that encodes the ECD amino acid sequence of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the

entire length of a nucleotide sequence (a) or (b).

14. An isolated or recombinant polypeptide, which polypeptide comprises a non-naturally-occurring amino acid sequence encoded by a nucleic acid comprising a polynucleotide sequence selected from the group of: (a) a polynucleotide sequence selected from SEQ ID NOS:1-21 and 95-142, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent or highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a), (b), or (c), wherein the nucleotide fragment encodes a polypeptide having a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1; (e) a polynucleotide sequence encoding a polypeptide, the polypeptide comprising an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and (f) a polynucleotide sequence encoding a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, which polynucleotide sequence has at least about 70% identity to at least one polynucleotide sequence of (a), (b), (c), or (d).

15. The isolated or recombinant polypeptide of claim 14, the polypeptide comprising an amino acid sequence of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

16. The isolated or recombinant polypeptide of claim 14, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

17. The isolated or recombinant polypeptide of claim 14 or 16, wherein the polypeptide induces T-cell proliferation.

18. The isolated or recombinant polypeptide of claim 14 or 16, wherein the polypeptide induces a T-cell proliferative response about equal to or greater than that of human B7-1.

19. An isolated or recombinant polypeptide comprising a amino acid sequence according to the formula: MGHTM-X6-W-X8-SLPPK-X14-PCL-X18-X19-X20-QLLVLT-X27-LFYFCSGITPKSVTKRVKETVMLSCDY-X55-TSTE-X60-LTSLRIYW-X69-KDSKMVLAILPGKVQVWPEYKNRTITDMNDN-X101-RIVI-X106-ALR-X110-SD-X113-GTYTCV-X120-QKP-X124-LKGAYKLEHL-X135-SVRLMIRADFPVP-X149-X150-X151-DLGNPSPNIRRLICS-X167-X168-X169-GFPRPHL-X177-WLENGEELNATNTT-X192-SQDP-X197-T-X199-LYMISSEL-X208-FNVTNN-X215-SI-X218-CLIKYGEL-X227-VSQIFPWSKPKQEPPIDQLPF-X249-VIIPVSGALVL-X261-A-X263-VLY-X267-X268-ACRH-X273-ARWKRTRRNEETVGTE RLSPIYLGSAQSSG (SEQ ID NO:284), or a subsequence thereof comprising an extracellular domain, wherein position X6 is Lys or Glu; position X8 is Arg or Gly; position X14 is Arg or Cys; position X18 is Trp or Arg; position X19 is Pro or Leu; position X20 is Ser or Pro; position X27 is Asp or Gly; position X55 is Asn or Ser; position X60 is Glu or Lys; position X69 is Gln or Arg; position X101 is Pro or Leu; position X106 is Leu or Gln; position X110 is Pro or Leu; position X113 is Lys or Ser; position X120 is Val or Ile; position X124 is Val or Asp; position X135 is Thr or Ala; position X149 is Thr, Ser, or del; position X150 is Ile or del; position X151 is Asn or Thr; position X167 is Thr or del; position X169 is Ser or del; position X169 is Gly or del; position X177 is Cys or Tyr; position X192 is Val or Leu; position X197 is Gly or Glu; position X199 is Glu or Lys; position X208 is Gly or Asp; position X215 is His or Arg; position X218 is Ala or Val; position X227 is Ser or Leu; position X249 is Trp, Leu, or Arg; position X261 is Ala or Thr; position X263 is Val, Ala, or Ile; position X267 is Arg or Cys; position X268 is Pro or Leu; and position X273 is Gly or Val.

20. The isolated or recombinant polypeptide of claim 19, which polypeptide comprises an extracellular domain amino acid sequence of any one of SEQ ID NOS:51-56, 58, 61, 66, 67, 174-179, 181, 185-187, 189, 192-194, 197, 199, 202, 205, 208, 215, 217, 220, and 285.

21. The isolated or recombinant polypeptide of claim 19, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

22. The isolated or recombinant polypeptide of claim 19 or 21, wherein the polypeptide induces T-cell proliferation.

23. The isolated or recombinant polypeptide of claim 19 or 21, wherein the polypeptide induces a T-cell proliferative response about equal to or greater than that of human B7-1.

24. The isolated or recombinant polypeptide of claim 19, comprising three or more of: Lys at position X6; Arg at position X8; Arg at position X14; Trp at position X18; Pro at position X19; Ser at position X20; Asp at position X27; Asn at position X55; Leu at position X106; Pro at position X110; Lys at position X113; Val at position X120; Val at position X124; Thr at position X135; Asn at position X151; Cys at position X177; Val at position X192; Gly at position X197; Glu at position X199; Gly at position X208; His at position X215; Ala at position X218; Trp at position X249; Ala at position X261; Val at position X263; Arg at position X267; Pro at position X268; and Gly at position X273.

25. The isolated or recombinant polypeptide of claim 24, comprising three or more of: Arg at position X8; Arg at position X14; Trp at position X18; Pro at position X19; Ser at position X20; Pro at position X110; Val at position X120; Val at position X124; Cys at position X177; Val at position X192; Gly at position X197; Glu at position X199; Gly at position X208; His at position X215; Ala at position X218; Trp at position X249; Ala at position X261; and Val at position X263.

26. The isolated or recombinant polypeptide of claim 25, comprising the extracellular domain amino acid sequence of SEQ ID NO:66 or SEQ ID NO:285.

27. The isolated or recombinant polypeptide of claim 25, comprising the entire amino acid sequence of SEQ ID NO:66 or SEQ ID NO:285.

28. An isolated or recombinant polypeptide comprising a subsequence of an amino acid sequence set forth in any of SEQ ID NOS:48-68, 174-182, 184-221, 283-285, and 290-293, wherein the subsequence is the extracellular domain of said amino acid sequence.

29. The isolated or recombinant polypeptide of claim 1, 14, 19, or 28, further comprising a **signal** peptide sequence.

30. The polypeptide of claim 29, wherein the **signal** peptide sequence is selected from the **signal** peptide sequence set forth in any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

31. The polypeptide of claim 30, further comprising a transmembrane domain amino acid sequence or a cytoplasmic domain amino acid sequence selected from the transmembrane domain amino acid sequence or the cytoplasmic domain amino acid sequence set forth in any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

32. The polypeptide of claim 1, 14, 19, or 28 comprising a soluble extracellular domain, wherein said polypeptide or ECD thereof binds CD28 and/or CTLA-4.

33. The polypeptide of claim 1, 14, 19, 28, 29, or 31, wherein the polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.

34. The polypeptide of claim 33, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

35. The polypeptide of claim 34, wherein the at least one Ig polypeptide comprises at least one human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

36. The polypeptide of claim 1, 14, 19, 28, 29, or 31, comprising a polypeptide purification subsequence.

37. The polypeptide of claim 36, wherein the polypeptide purification subsequence is selected from: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

38. The polypeptide of claim 1, 14, 19, 28, 29, or 31 comprising a modified amino acid.

39. The polypeptide of claim 38, wherein the modified amino acid is selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

40. A composition comprising at least one polypeptide of claim 38 and a pharmaceutically acceptable excipient.

41. A composition comprising at least one polypeptide of claim 1, 14, 19, 28, 29, or 31 and a pharmaceutically acceptable excipient.

42. A composition comprising: an isolated or recombinant polypeptide comprising an amino acid sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, 290-293, or a costimulatory fragment thereof, wherein said polypeptide or costimulatory fragment has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response equal to or greater than that induced by human B7-1, and a carrier.

43. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS:1-21 and 95-142, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); and (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a), (b), or (c), wherein the nucleotide fragment encodes a polypeptide having a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 or a polypeptide having an ability to induce a T cell proliferation response that is about equal to or greater than that induced by human B7-1.

44. An isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding a polypeptide, wherein the encoded polypeptide comprises an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293 and is a non naturally-occurring amino acid sequence.

45. The nucleic acid of claim 44, wherein the encoded polypeptide is substantially identical over at least about 175 contiguous amino acid residues of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

46. An isolated or recombinant nucleic acid comprising a nucleotide sequence coding for a polypeptide comprising the amino acid sequence set forth in any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a subsequence thereof, wherein the subsequence comprises at least one of: the **signal sequence** of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide, and wherein the amino acid sequence or subsequence is a non naturally-occurring sequence.

47. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response equal to or greater than that of human B7-1.

48. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

49. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

50. The nucleic acid of claim 43, 44, 46, or 49, wherein the encoded polypeptide induces T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

51. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

52. The nucleic acid of claim 43, 44, or 46, wherein the nucleic acid encodes a fusion protein comprising at least one additional amino acid sequence.

53. The nucleic acid of claim 52, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

54. The nucleic acid of claim 53, wherein the at least one Ig polypeptide comprises at least one human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

55. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide comprises a **signal sequence**.

56. The nucleic acid of claim 43, 44, 46, or 49, wherein the encoded polypeptide comprises a precursor peptide.

57. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide comprises an epitope tag sequence.

58. A cell comprising the nucleic acid of claim 43, 44, 46, or 49.

59. The cell of claim 58, wherein the cell expresses a polypeptide encoded by the nucleic acid.

60. A vector comprising the nucleic acid of claim 43, 44, 46, or 49.

61. The vector of claim 60, wherein the vector comprises a plasmid, a cosmid, a phage, a virus, a virus-like particle, or a fragment of a virus.

62. The vector of claim 60, wherein the vector is an expression vector.

63. The expression vector of claim 62, wherein the nucleic acid is operably linked to a promoter.

64. The expression vector of claim 62, further comprising a polynucleotide sequence encoding an antigen.

65. The expression vector of claim 64, wherein the antigen is a cancer antigen.

66. The expression vector of claim 64, wherein the nucleic acid is operably linked to first promoter and the polynucleotide sequence encoding the antigen is operably linked to a second promoter.

67. The expression vector of claim 65, wherein the cancer antigen is EpCam/KSA or a mutant or variant thereof.

68. The expression vector of claim 67, wherein the expression vector comprises the vector shown in FIG. 22B.

69. A host cell comprising the vector of claim 60.

70. A composition comprising the nucleic acid of claim 43, 44, 46, or 49 and an excipient.

71. The composition of claim 70, wherein the excipient is a pharmaceutically acceptable excipient.

72. A composition of matter comprising at least one nucleic acid of claim 43, 44, 46, or 49.

73. The composition of claim 72, wherein the composition comprises a library comprising at least about 2, 5, 10, 50 or more nucleic acids.

74. A composition produced by cleaving at least one nucleic acid of claim 43, 44, or 46.

75. The composition of claim 74, wherein the cleaving comprises mechanical, chemical, or enzymatic cleavage.

76. The composition of claim 75, wherein the enzymatic cleavage comprises cleavage with a restriction endonuclease, an RNAse, or a DNAse.

77. A composition produced by a process comprising incubating at least one nucleic acid of claim 43, 44, or 46 in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase.

78. The composition of claim 77, wherein the nucleic acid polymerase is a thermostable polymerase.

79. An isolated or recombinant nucleic acid encoding a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, produced by mutating or recombining at least one nucleic acid of claim 43, 44, or 46.

80. An isolated or recombinant polypeptide comprising an amino acid sequence having at least about 95% identity to at least about one of SEQ ID NOS:69-92, 222-252, 286-289, or a subsequence thereof comprising an extracellular domain, wherein said amino acid sequence (a) is a non naturally-occurring amino acid sequence, and (b) comprises at least one of: Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; or Thr at position 279, wherein the position number corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO:278), wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response that is equal to or less than that of human B7-1.

81. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to at least one of SEQ ID NOS:69-92, 222-252, 286-289, or a subsequence thereof comprising an extracellular domain, said amino acid sequence comprising at least one of: Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; and Thr at position 279, wherein the position number corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

82. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to at least one of SEQ ID NOS:69-92, 222-252, and 286-289, said amino acid sequence comprising at least one of: Gly at position 2; Gly at position 8; Cys at position 27; His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; Arg at position 219; Thr at position 250; Arg at position 266; Lys at position 275; and Ser at position 276, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

83. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to an extracellular domain of at least one of SEQ ID NOS:69-92, 222-252, and 286-289, said amino acid sequence comprising at least one of: His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; and Arg at position 219, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

84. The isolated or recombinant polypeptide of claim 83, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to an extracellular domain of at least one of SEQ ID NOS:69-92, 222-252, 286-289, said sequence comprising at least two of: His at

position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; and Arg at position 219, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

85. The isolated or recombinant polypeptide of claim 84, wherein said polypeptide comprises an extracellular domain of any one of SEQ ID NOS:81, 85, 86, 88, 90, and 91.

86. The isolated or recombinant polypeptide of claim 80, which polypeptide comprises an extracellular domain of any one of SEQ ID NOS:69-92, 222-252, and 286-289.

87. The isolated or recombinant polypeptide of claim 80, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:69-92, 222-252, and 286-289.

88. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

89. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

90. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has a decreased or a lowered binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

91. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide inhibits T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

92. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide inhibits T-cell proliferation.

93. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide induces a T-cell response less than that of human B7-1.

94. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

95. The isolated or recombinant polypeptide of claim 84 or 91, which polypeptide comprises an extracellular domain amino acid sequence of any one of SEQ ID NOS:69-92 and 222-247.

96. The isolated or recombinant polypeptide of claim 80 or 91, which polypeptide comprises an extracellular domain amino acid sequence encoded by a coding polynucleotide sequence, the coding polynucleotide sequence selected from the group: (a) an extracellular domain coding sequence of a polynucleotide sequence selected from any of SEQ ID NOS:22-45 and 143-173; (b) a polynucleotide sequence that encodes the extracellular domain of a polypeptide selected from any of SEQ ID NOS:69-92, 222-252, and 286-289; and (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a polynucleotide sequence (a) or (b).

97. An isolated or recombinant polypeptide comprising an amino acid sequence that differs from a primate B7-1 amino acid sequence in at least one mutation selected from: Ser 12 Pro; Leu 25 Met; Gly 27 Cys; Ser 29 Pro; Lys 40 Arg; His 52 Leu; Tyr 65 His; Glu 122 Asp; Glu 129 Lys; Thr 135 Met; Thr 164 Ala; Ser 174 Phe; Glu 196 Gly; Ala 199 Thr; Thr 210 Ala; Lys 219 Arg; Thr 234 Pro; Asp 241 Asn; Val 254 Ala; Arg 275 Lys; Arg 276 Ser; or Arg 279 Thr; the mutation being indicated comprising a mutation relative to human B7-1 with the amino acid sequence shown in SEQ ID NO:278, wherein said amino acid sequence does not occur in nature, and wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

98. The isolated or recombinant polypeptide of claim 97, wherein said amino acid sequence differs from said primate B7-1 sequence in at least two of said mutations.

99. The isolated or recombinant polypeptide of claim 97, wherein said primate B7-1 is human B7-1 (SEQ ID NO:278).

100. The isolated or recombinant polypeptide of claim 99, wherein said sequence differs from the human B7-1 sequence in at least two of said mutations.

101. An isolated or recombinant polypeptide comprising an amino acid sequence, said amino acid sequence having at least about 75% identity to at least one polypeptide sequence of SEQ ID NOS:263-272, or a subsequence thereof comprising the extracellular domain, wherein said amino acid sequence is not a naturally-occurring amino acid sequence, and wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

102. An isolated or recombinant polypeptide, which polypeptide comprises a non naturally-occurring amino acid sequence encoded by a nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS:22-45, 143-173, 253-262, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:69-92, 222-247, 263-272, 286-289, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide having a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1; (e) a polynucleotide sequence encoding a polypeptide comprising an amino acid sequence that is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS:69-92, 222-247, 263-272, 286-289, and (f) a polynucleotide sequence encoding a polypeptide that has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, which polynucleotide sequence has at least about 70% identity to at least one polynucleotide sequence of (a), (b), (c), or (d).

103. The isolated or recombinant polypeptide of claim 102, the polypeptide comprising an amino acid sequence of any one of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

104. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

105. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide inhibits T-cell proliferation.

106. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide induces a T-cell response less than that of human B7-1.

107. An isolated or recombinant polypeptide comprising an amino acid sequence according to the formula: MGHTIRRQGTSP-X12-KCPYLKFFQLLV-X25-ACL-X29-HILCSGVHIHVT-X40-EVKEVATLSCGLNVSVEELAQTRIHWQKEKKMVLTM MSGDMNIWPEYKNRTIFDITNNLSIVILALRPSDEGTYECVVLKY-X122-KDAFKR-X129-HLAEVMLSVKAD FPTPSITDFEIPPSNIRRIICS-X164-SGGFPEPHLFWLENGEELNAINTVSQDPET-X196-LYTVSSKLDFNM TANHSFMCLI-X219-YGHLRVNQTFNWNTPKQEHIFP-X241-NLLPSWA ITLISANGIFVICCLTYRFAPRCRERKSNETLRRESVCPV (SEQ ID NO:287), or a subsequence thereof comprising the extracellular domain, wherein position X12 is Ser or Pro; position X25 is Leu or Met; position X29 is Ser or Pro; position X40 is Lys or Arg; position X122 is Glu or Asp; position X129 is Glu or Lys; position X164 is Thr or Ala; position X196 is Glu or Gly; position X219 is Lys or Arg; and position X241 is Asp or Asn.

108. The isolated or recombinant polypeptide of claim 107, which polypeptide comprises the extracellular domain of SEQ ID NO:288 or SEQ ID NO:289.

109. The isolated or recombinant polypeptide of claim 107, comprising the sequence SEQ ID NO:288 or SEQ ID NO:289.

110. The isolated or recombinant polypeptide of claim 107, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

111. The isolated or recombinant polypeptide of claim 107 or 110,

wherein the polypeptide inhibits T-cell proliferation.

112. The isolated or recombinant polypeptide of claim 107 or 110, wherein the polypeptide induces a T-cell response less than that of human B7-1.

113. An isolated or recombinant polypeptide comprising a subsequence of an amino acid sequence set forth in any of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289, wherein the subsequence is the extracellular domain of said amino acid sequence.

114. The isolated or recombinant polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a **signal sequence**.

115. The polypeptide of claim 114, wherein the **signal sequence** is selected from the **signal sequence** set forth in any of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

116. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a transmembrane domain sequence or a cytoplasmic domain sequence, selected from the transmembrane domain sequence or the cytoplasmic domain sequence set forth in any of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

117. The polypeptide of claim 80, 97, 101, 102, 107, or 113 comprising a soluble extracellular domain.

118. The polypeptide of claim 80, 97, 101, 102, 107, or 113, wherein the polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.

119. The polypeptide of claim 118, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

120. The polypeptide of claim 119, wherein the at least one Ig polypeptide comprises a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

121. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a polypeptide purification subsequence.

122. The polypeptide of claim 121, wherein the polypeptide purification subsequence is selected from: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

123. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a modified amino acid.

124. The polypeptide of claim 123, wherein the modified amino acid is selected from the group consisting of: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

125. A composition comprising at least one polypeptide of claim 124 and a pharmaceutically acceptable excipient.

126. A composition comprising at least one polypeptide of claim 80, 97, 101, 102, 107, or 113, and a pharmaceutically acceptable excipient.

127. A composition comprising: an isolated or recombinant polypeptide comprising the amino acid sequence of SEQ ID NOS:69-92, 222-247, 263-272, 286-289, or a costimulatory fragment thereof, wherein said costimulatory fragment has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, and a carrier.

128. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS:22-45, 143-173, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:69-92, 222-247, 286-289, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); and (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a), (b), or (c); wherein (c) or (d) encodes a polypeptide having a non naturally-occurring amino acid sequence comprising at least one of: Gly at position 2; Thr at

position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; and Thr at position 279, wherein the number of the amino acid position corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO:278), and wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1 and/or an ability to induce a T cell proliferation response that is about equal to or greater than that induced by human B7-1.

129. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NOS:253-262, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:263-272, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b) and encodes a polypeptide having a non naturally-occurring sequence; and (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide having (i) a non naturally-occurring sequence and (ii) a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

130. An isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding a polypeptide, the encoded polypeptide comprising an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

131. The nucleic acid of claim 44, wherein the encoded polypeptide is substantially identical over at least about 200 contiguous amino acid residues of any one of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

132. An isolated or recombinant nucleic acid comprising a nucleotide sequence coding for a polypeptide comprising the amino acid sequence set forth in any of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289, or a subsequence thereof, wherein the subsequence comprises at least one of: the **signal** peptide sequence of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide, and wherein the amino acid sequence or subsequence is a non naturally-occurring amino acid sequence.

133. The nucleic acid of claim 128, 129, 130, or 132, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

134. The nucleic acid of claim 128, 129, 130, or 132, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

135. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

136. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide inhibits T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

137. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

138. The nucleic acid of claim 128, 129, 130, or 132, wherein the nucleic acid encodes a fusion protein comprising at least one additional

amino acid sequence.

139. The nucleic acid of claim 138, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

140. The nucleic acid of claim 139, wherein the at least one Ig polypeptide comprises a human IgG polypeptide comprising an Fe hinge, a CH2 domain, and a CH3 domain.

141. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises a **signal** peptide sequence.

142. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises a precursor peptide.

143. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises an epitope tag sequence.

144. A cell comprising the nucleic acid of claim 128, 129, 130, or 132.

145. The cell of claim 144, wherein the cell expresses a polypeptide encoded by the nucleic acid.

146. A vector comprising the nucleic acid of claim 128, 129, 130, 132, 133, or 136.

147. The vector of claim 146, wherein the vector comprises a plasmid, a cosmid, a phage, a virus, a virus-like particle, or a fragment of a virus.

148. The vector of claim 146, wherein the vector is an expression vector.

149. The expression vector of claim 148, wherein the nucleic acid is operably linked to a promoter.

150. The expression vector of claim 149, further comprising a polynucleotide sequence encoding at least one Ig polypeptide or fragment thereof.

151. The expression vector of claim 150, wherein the at least one Ig polypeptide is a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

152. The expression vector of claim 150, wherein the promoter is a CMV promoter.

153. The expression vector of claim 150, further comprising a BGH polyA sequence.

154. A host cell comprising the vector of claim 146.

155. A composition comprising the nucleic acid of claim 128, 129, 130, or 132, and an excipient.

156. The composition of claim 155, wherein the excipient is a pharmaceutically acceptable excipient.

157. A composition of matter comprising at least one nucleic acid of claim 128, 129, 130, 132, 133, or 136.

158. The composition of claim 157, wherein the composition comprises a library comprising at least about 2, 5, 10, 50 or more nucleic acids.

159. A composition produced by cleaving at least one nucleic acid of claim 128, 129, 130, or 132.

160. The composition of claim 159, wherein the cleaving comprises mechanical, chemical, or enzymatic cleavage.

161. The composition of claim 160, wherein the enzymatic cleavage comprises cleavage with a restriction endonuclease, an RNAse, or a DNAse.

162. A composition produced by a process comprising incubating at least one nucleic acid of claim 128, 129, 130, or 132 in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase.

163. The composition of claim 162, wherein the nucleic acid polymerase

is a thermostable polymerase.

164. An isolated or recombinant nucleic acid encoding a polypeptide that has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, produced by mutating or recombining at least one nucleic acid of claim 128, 129, 130, or 132.

165. An isolated or recombinant polypeptide comprising an amino acid sequence according to the formula: MGHTMKWGSLPPKRPCLWLSQLLVLTGLFYFCSGIT PK SVTKRVKETVM-X50-SCDY-X55-X56-STEELTSLRIYWQKDSKMVL AILPGKVQVWPEYKNRTITD MNDNPRIVILALRLSD-X113-GTYTCV-X120-QK-X123-X124-X125-X126-G-X128-X129-X130-X13 1-EHL-X135-SV-X138-L-X140-IRADFPVPSITDIGHPAPNVK RIRCSASG-X170-FPEPRLAWMEDGEEL NAVNTTV-X193-X194-X195-LDTELYSVSSELD-X209-N-X211-TNNHSIVCLIKYGELSVSQIFPWSKPK QEPPIDQLPFWVI-X252-X253-VSGALVLTAVVLYCLACRHVAR (SEQ ID NO:290), or a subsequence thereof comprising the extracellular domain, wherein position X50 is Leu or Pro; position X55 is Asn or Ser; position X56 is Ala or Thr; position X113 is Ser or Lys; position X120 is Ile or Val; position X123 is Pro or deleted; position X124 is Val, Asn, or Asp; position X125 is Leu or Glu; position X126 is Lys or Asn; position X128 is Ala or Ser; position X129 is Tyr or Phe; position X130 is Lys or Arg; position X131 is Leu or Arg; position X135 is Ala or Thr; position X138 is Arg or Thr; position X140 is Met or Ser; position X170 is Asp or Gly; position X193 is Asp or is deleted; position X194 is Gln or is deleted; position X195 is Asp or is deleted; position X211 is Val or Ala; position X252 is Ile or Val; and position X253 is Leu or Pro.

166. The isolated or recombinant polypeptide of claim 165, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:59, 62, 180, 184, 188, 195, 196, 200, 201, 204, 211, 213, 219, and 291.

167. An isolated or recombinant polypeptide comprising an amino acid sequence according to the formula: MGHTMKWG-X9-LPPKRPCLWLSQLLVLTGLFYFCSG-X35-TPKSVTKRV KETVMLSCDY-X55-TSTEELTSLRIYWQKDSKMVLAILPGKVQVW PEYKNRTITDMNDNPRIVILALR-X110-SDSGTYTCVIQKP-X124-LKGAYKLEHL-X135-SVRLMIRADFPVPTINDLGNPSPNIRRLICSTSGGFP RPHLYWLENG-X183-ELNATNTT-X192-SQDPETKLYMISSELDFN-X211-TSN-X215-X216-X217-LCLVKYGDLTVSQ-X231-FYWQESKPTPSANQHLTWTIIIPVSAFGISVIIAVI LTCLTCRNAAIRRQRRENEV-X288-M-X290-SCSQSP (SEQ ID NO:292), or a subsequence thereof comprising the extracellular domain, wherein position X9 is Thr or Ser; position X35 is Ile or Thr; position X55 is Asn or Ser; position X110 is Leu or Pro; position X124 is Asp or Val; position X135 is Thr or Ala; position X183 is Lys or Glu; position X192 is Leu or Val; position X211 is Met or Thr; position X215 is His or is deleted; position X216 is Ser or is deleted; position X217 is Phe or is deleted; position X231 is Thr or Ser; position X288 is Lys or Glu; position X290 is Glu or Gln, and wherein said sequence is a non naturally-occurring sequence.

168. The isolated or recombinant polypeptide of claim 167, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:48, 182, 183, 212, 214, 216, 218, 221, and 293.

169. An isolated or recombinant polypeptide comprising the sequence SEQ ID NO:93, SEQ ID NO:94, or a subsequence thereof, wherein the subsequence comprises at least one of: the **signal** peptide of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide.

170. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: (a) a polynucleotide sequence selected from SEQ ID NO:46, SEQ ID NO:47, or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO:93, SEQ ID NO:94, or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence encoding a subsequence of a polypeptide selected from SEQ ID NO:93, SEQ ID NO:94, or a complementary polynucleotide sequence thereof, wherein the subsequence comprises at least one of: the **signal** peptide of said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide.

171. A polypeptide which is specifically bound by a polyclonal antisera raised against at least one antigen, the at least one antigen comprising the sequence SEQ ID NOS:48-94, 174-252, 263-272, 283-293, or a fragment thereof, wherein the antisera is subtracted with a polypeptide encoded by one or more of GenBank Nucleotide Accession Nos: A92749, A92750,

AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

172. An antibody or antisera produced by administering the polypeptide of claim 1, 80, 101, or 169 to a mammal, which antibody or antisera specifically binds at least one antigen, the at least one antigen comprising a polypeptide comprising one or more of the amino acid sequences SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, which antibody or antisera does not specifically bind to a polypeptide encoded by one or more of GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

173. An antibody or antisera which specifically binds a polypeptide, the polypeptide comprising an amino acid sequence selected from: SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, wherein the antibody or antisera does not specifically bind to a polypeptide encoded by one or more of GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

174. A method of producing a polypeptide, the method comprising: (a) introducing into a population of cells a nucleic acid of claim 43, 46, 128, 129, 132, or 170, the nucleic acid operatively linked to a regulatory sequence effective to produce the encoded polypeptide; (b) culturing the cells in a culture medium to produce the polypeptide; and (c) isolating the polypeptide from the cells or from the culture medium.

175. A method of producing a polypeptide, the method comprising (a) introducing into a population of cells a recombinant expression vector comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170; (b) culturing the cells in a culture medium to produce the polypeptide encoded by the expression vector; and (c) isolating the polypeptide from the cells or from the culture medium.

176. A method of producing a polypeptide, the method comprising: (a) introducing into a population of cells a recombinant expression vector comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170; (b) administering the expression vector into a mammal; and (c) isolating the polypeptide from the mammal or from a byproduct of the mammal.

177. A method of inducing T-cell proliferation, the method comprising: contacting a population of T cells with a polypeptide of claim 1, 80, 101, or 169, thereby inducing proliferation of the T cells.

178. A method of inhibiting T-cell proliferation, the method comprising: contacting a population of T cells with a polypeptide of claim 1, 80, 101, or 169, thereby inhibiting proliferation of the T cells.

179. A method of modifying T-cell proliferation, the method comprising: contacting a population of T cells with a polypeptide of claim 1, 80, 101, or 169, thereby modifying proliferation of the T cells.

180. A method of modifying T-cell activation, the method comprising: contacting a population of T cells with a polypeptide of claim 1, 80, 101, or 169, thereby modifying activation of the T cells.

181. The method of claim 177, 178, 179, or 180 wherein the T cells are in culture.

182. A method of treating an autoimmune disorder or medical condition in a subject, the method comprising: administering to the subject an

effective amount of the polypeptide of claim 1, 80, 101, or 169.

183. A method of treating an autoimmune disorder or medical condition in a subject, the method comprising: administering to the subject an effective amount of an expression vector comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170

184. The method of claim 182, wherein the autoimmune disorder or medical condition is selected from the group comprising: multiple sclerosis, rheumatoid arthritis, lupus erythematosus, psoriasis, and type I diabetes.

185. The method of claim 182, wherein the medical condition comprises an allogeneic or xenogeneic graft or transplant.

186. A method of treating a medical disorder in a subject, the method comprising: administering to the subject an effective amount of the polypeptide of claim 1, 80, 101, or 169.

187. The method of claim 186, wherein the medical disorder comprises: cancer, viral infection (e.g. HIV), or bacterial infection.

188. In a method of treating a disorder treatable by administration of a co-stimulatory molecule to a subject, an improved method comprising: administering to the subject an effective amount of the polypeptide of claim 1, 80, 101, or 169.

189. The method of claim 188, wherein the disorder treatable by administration of a co-stimulatory molecule is selected from the group comprising: sclerosis, rheumatoid arthritis, lupus erythematosus, psoriasis, type I diabetes, allogeneic grafts, xenogeneic grafts, cancer, viral infection, and bacterial infection.

190. A method of recombination, the method comprising recursively recombining one or more nucleic acids of claim 43, 46, 128, 129, 132, or 170, with at least one additional nucleic acid.

191. The method of claim 190, wherein the at least one additional nucleic acid encodes a co-stimulatory homologue or subsequence thereof.

192. The method of claim 190, wherein the recursive recombination produces at least one library of recombinant co-stimulatory homologue nucleic acids.

193. A nucleic acid library produced by the method of claim 192.

194. A population of cells comprising the library of claim 193.

195. A recombinant co-stimulatory homologue nucleic acid produced by the method of claim 191.

196. A cell comprising the nucleic acid of claim 195.

197. The method of claim 190, wherein the recursive recombination is performed in vitro.

198. The method of claim 190, wherein the recursive recombination is performed in vivo.

199. A method of producing a modified co-stimulatory nucleic acid homologue comprising mutating a nucleic acid of claim 43, 46, 128, 129, 132, or 170.

200. The modified co-stimulatory homologue nucleic acid homologue produced by the method of claim 199.

201. A computer or computer readable medium comprising a database comprising a sequence record comprising one or more character strings corresponding to a nucleic acid or polypeptide sequence selected from SEQ ID NOS:1-272 and 283-293.

202. An integrated system comprising a computer or computer readable medium comprising a database comprising one or more sequence records, each of said one or more sequence records comprising one or more character strings corresponding to a nucleic acid or polypeptide sequence selected from SEQ ID NOS:1-272 and 283-293, the integrated system further comprising a user input interface allowing a user to selectively view one or more sequence records.

203. The integrated system of claim 202, the computer or computer readable medium comprising an alignment instruction set which aligns the one or more character strings with one or more additional character strings corresponding to a nucleic acid or polypeptide sequence.

204. The integrated system of claim 203, wherein the instruction set comprises one or more of: a local homology comparison determination, a homology alignment determination, a search for similarity determination, and a BLAST determination.

205. The integrated system of claim 203, further comprising a user readable output element which displays an alignment produced by the alignment instruction set.

206. The integrated system of claim 202, the computer or computer readable medium further comprising an instruction set which translates one or more nucleic acid sequences, each of said one or more nucleic acid sequences comprising a sequence selected from SEQ ID NOS:1-47, 95-173, and 253-262, into an amino acid sequence.

207. The integrated system of claim 202, the computer or computer readable medium further comprising an instruction set for reverse-translating at least one amino acid sequence comprising a sequence selected from SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, into a nucleic acid sequence.

208. The integrated system of claim 207, wherein the instruction set selects the nucleic acid sequence by applying a codon usage instruction set or an instruction set which determines sequence identity to a test nucleic acid sequence.

209. A method of using a computer system to present information pertaining to at least one of a plurality of sequence records stored in a database, said sequence records each comprising one or more character strings corresponding to SEQ ID NOS:1-272 and 283-293, the method comprising: (a) determining a list of one or more character strings corresponding to one or more of SEQ ID NOS:1-272 and 283-293, or a subsequence thereof; (b) determining which character strings of said list are selected by a user; and (c) displaying the selected character strings, or aligning the selected character strings with an additional character string.

210. The method of claim 209, further comprising displaying an alignment of the selected character string with the additional character string.

211. The method of claim 209, further comprising displaying the list.

212. A nucleic acid which comprises a unique subsequence in a nucleic acid selected from SEQ ID NOS:1-47, 95-173, and 253-262, wherein the unique subsequence is unique as compared to a nucleic acid corresponding to any of GenBank Nucleotide Accession No.: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

213. A polypeptide which comprises a unique subsequence in a polypeptide selected from: SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, wherein the unique subsequence is unique as compared to a polypeptide encoded by any of GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

214. A target nucleic acid which, but for the degeneracy of the genetic code, hybridizes under stringent conditions to a unique coding oligonucleotide which encodes a unique subsequence in a polypeptide selected from: SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, wherein the unique subsequence is unique as compared to a polypeptide encoded by any of GenBank Nucleotide Accession No.: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829,

AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

215. The nucleic acid of claim 214, wherein the stringent conditions are selected such that a perfectly complementary oligonucleotide to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5× higher **signal** to noise ratio than for hybridization of the perfectly complementary oligonucleotide to a control nucleic acid corresponding to any of GenBank Nucleotide Accession No.: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950, wherein the target nucleic acid hybridizes to the unique coding oligonucleotide with at least about a 2× higher **signal** to noise ratio as compared to hybridization of the control nucleic acid to the coding oligonucleotide.

216. A method of therapeutic or prophylactic treatment of a disease or disorder in a subject in need of such treatment, comprising: administering to the subject at least one polypeptide of claim 1, 80, 101, or 169 or at least one nucleic acid of claim 43, 46, 128, 129, 132, or 170, and at least one immunogen specific for said disease or disorder, wherein the combined amount of the at least one polypeptide or at least one nucleic acid and the at least one immunogen is effective to prophylactically or therapeutically treat said disease or disorder.

217. The method of claim 216, wherein the at least one polypeptide or nucleic acid is present in an amount sufficient to enhance, diminish, modulate or modify an immune response induced by the at least one immunogen.

218. The method of claim 216, wherein a composition comprising the at least one polypeptide or nucleic acid, the immunogen, and a pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat said disease or disorder.

219. The method of claim 216, wherein the subject is a mammal.

220. The method of claim 219, wherein the mammal is a human.

221. The method of claim 216, wherein the polypeptide is administered in vivo to the subject.

222. The method of claim 216, wherein the polypeptide is administered in vitro or ex vivo to one or more cells of the subject.

223. A method of modulating an immune response in a subject, comprising: administering to the subject a polynucleotide comprising a nucleic acid sequence of claim 43, 46, 128, 129, 132, or 170, operably linked to a promoter sequence that controls the expression of said nucleic acid sequence, said polynucleotide being present in an amount sufficient that uptake of said polynucleotide into one or more cells of the subject occurs and sufficient expression of said nucleic acid sequence results to produce an amount of a polypeptide effective to modulate an immune response.

224. The method of claim 223, further comprising administering to the subject an antigen specific for the disease or disorder, wherein the polynucleotide is administered to the subject in an amount sufficient to modulate the immune response induced in the subject by the antigen.

225. The method of claim 223, wherein the polynucleotide further comprises a nucleotide sequence encoding for an antigen.

226. The method of claim 223, wherein the polynucleotide further comprises at least one additional nucleotide sequence encoding a cytokine, adjuvant, co-stimulatory molecule, or at least one additional nucleotide sequence comprising a promoter.

227. The method of claim 223, wherein the subject is a mammal.

228. The method of claim 227, wherein the mammal is a human.

229. The method of claim 223, wherein said polynucleotide comprises a vector.

230. A method of treating a disease or disorder in a subject in need of such treatment, comprising: administering to the subject at least one polypeptide of claim 1, 80, 101, or 169 or at least one nucleic acid of claim 43, 46, 128, 129, 132, or 170 in an amount effective to treat said disease or disorder.

231. A method of therapeutic or prophylactic treatment of a disease or disorder in a subject in need of such treatment, comprising: administering to the subject a polypeptide of claim 32 or 117 and an immunogen specific for said disease or disorder, wherein the combined amount of polypeptide and immunogen is effective to prophylactically or therapeutically treat said disease or disorder.

232. The method of claim 231, wherein the polypeptide is present in an amount sufficient to enhance, diminish or modify an immune response induced by the immunogen.

233. The method of claim 231, wherein a composition comprising the polypeptide, the immunogen, and a pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat the disease or disorder.

234. The method of claim 231, wherein the subject is a mammal.

235. The method of claim 234, wherein the mammal is a human.

236. The method of claim 231, wherein the polypeptide is administered in vivo to the subject.

237. The method of claim 231, wherein the polypeptide is administered in vitro or ex vivo to one or more cells of the subject.

238. A method of treating a disease or disorder in a subject in need of such treatment, comprising: administering to the subject a polypeptide of claim 58 in an amount effective to treat the disease or disorder.

239. The isolated or recombinant polypeptide of claim 165, comprising three or more of: Leu at position X50; Asn at position X55; Ala at position X56; Ser at position X113; Ile at position X120; Pro at position X123; Val at position X124; Leu at position X125; Lys at position X126; Ala at position X128; Tyr at position X129; Lys at position X130; Leu at position X131; Ala at position X135; Arg at position X138; Met at position X140; Asp at position X170; Asp at position X193; Asp at position X194; Asp at position X195; Val at position X211; Ile at position X252; and Leu at position X253.

240. The isolated or recombinant polypeptide of claim 167, comprising three or more of: Thr at position X9; Ile at position X35; Asn at position X55; Leu at position X110; Asp at position X124; Thr at position X135; Lys at position X183; Leu at position X192; Met at position X211; His at position X215; Ser at position X216; Phe at position X217; Thr at position X231; Lys at position X288; and Glu at position X290.

241. A method of modulating or altering a T-cell response specific to an antigen in a subject, the method comprising administering to the subject at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 174-252, 263-272 and 283-293 or fragment thereof, and a polynucleotide sequence encoding the antigen or antigenic fragment thereof, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to modulate or alter a T cell response.

242. The method of claim 241, wherein the at least one polynucleotide sequence encoding a polypeptide comprises a polynucleotide sequence selected from any of SEQ ID NOS:1-47, 95-173, and 253-262.

243. The method of claim 241, wherein the polypeptide or fragment thereof interacts with or binds a T cell surface receptor.

244. The method of claim 241, wherein the T-cell response is enhanced.

245. The method of claim 244, wherein the enhanced T cell response is sufficient to eliminate cells bearing the antigen or antigenic fragment thereof.

246. The method of claim 241, wherein the T-cell response is suppressed or inhibited.

247. The method of claim 241, wherein the antigen or antigenic fragment thereof is an antigen or antigenic fragment thereof of an infectious agent or a cancer.

248. The method of claim 244, wherein the polypeptide comprises SEQ ID NO:66 or the extracellular domain amino acid sequence thereof.

249. The method of claim 245, wherein the polypeptide comprises SEQ ID NO:86 or the extracellular domain amino acid sequence thereof.

250. The method of claim 244, wherein the at least one polynucleotide sequence encoding a NCSM polypeptide or fragment thereof is operably linked to a promoter in a first vector.

251. The method of claim 250, wherein the at least one polynucleotide sequence encoding the antigen or antigenic fragment thereof is operably linked to a promoter in the first vector.

252. The method of claim 250, wherein the at least one polynucleotide sequence encoding the antigen or antigenic fragment thereof is operably linked to a promoter in the a second vector.

253. A vector comprising at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 174-252, 263-272 and 283-293 or fragment thereof, and a polynucleotide sequence encoding the antigen or antigenic fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, and wherein each of the at least one polynucleotide sequences is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

254. The vector of claim 253, wherein the at least one polynucleotide sequence encoding a polypeptide comprises a polynucleotide sequence of any of SEQ ID NOS:1-47, 95-173, and 253-262.

255. The vector of claim 253, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to enhance a T cell response such that cells expressing the antigen or antigenic fragment thereof are eliminated.

256. The vector of claim 253, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to inhibit a T cell response.

257. A vector comprising at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 174-252, 263-272 and 283-293 or fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, wherein the at least one polynucleotide sequence is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

258. A method of modulating or altering an immune response in a subject, the method comprising introducing into cells of a tumor of the subject at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 174-252, 263-272 and 283-293 or fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, and wherein the at least one polynucleotide sequence is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

259. An isolated or recombinant polypeptide comprising an amino acid sequence corresponding to an extracellular domain, wherein said amino acid sequence has at least about 92% amino acid sequence identity to the amino acid sequence corresponding to the extracellular domain of SEQ ID NO:66, and wherein said polypeptide has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

260. The isolated or recombinant polypeptide of claim 259, further comprising at least one further amino acid sequence corresponding to a **signal** peptide.

261. The isolated or recombinant polypeptide of claim 260, further comprising at least one further amino acid sequence corresponding to a transmembrane domain or a cytoplasmic domain.

262. The isolated or recombinant polypeptide of claim 259, wherein said extracellular domain amino acid sequence has at least about 95% amino acid sequence identity to an extracellular domain amino acid sequence of SEQ ID NO:66, and wherein said polypeptide has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, and/or an ability to induce a T cell proliferation response at least about equal to or greater than the T cell proliferation response induced by human B7-1.

263. An isolated or recombinant nucleic acid comprising a nucleotide sequence selected from the group of: (a) a nucleotide sequence that encodes an extracellular domain (ECD), said nucleotide sequence comprising an ECD coding subsequence of a polynucleotide sequence selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding an ECD, said ECD comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof; and (c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 and/or an ability to induce a T cell proliferation response equal to or greater than that induced by human B7-1.

264. The isolated or recombinant nucleic acid of claim 263, wherein the nucleotide sequence of (c) hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) and encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

265. The isolated or recombinant nucleic acid of claim 264, further comprising at least a second nucleotide sequence that encodes a **signal** peptide, wherein said second nucleotide sequence is selected from the group of: (a) a nucleotide sequence comprising a **signal** peptide coding subsequence of a polynucleotide sequence selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a **signal** peptide, said **signal** peptide comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof; (c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1; and (d) a nucleotide sequence encoding a **signal** peptide of a B7-1 polypeptide.

266. The isolated or recombinant nucleic acid of claim 265, wherein the nucleotide sequence of (c) hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) and encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response about equal to or greater than that induced by human B7-1.

267. The isolated or recombinant nucleic acid of claim 265, further comprising at least a third nucleotide sequence encoding a transmembrane domain selected from the group of: (a) a nucleotide sequence comprising a transmembrane domain coding subsequence of a polynucleotide sequence selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a transmembrane domain, said transmembrane domain comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof; (c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1;

and (d) a nucleotide sequence that encodes a transmembrane domain of a B7-1 polypeptide.

268. The isolated or recombinant nucleic acid of claim 267, further comprising at least a fourth nucleotide sequence encoding a cytoplasmic domain selected from the group of: (a) a nucleotide sequence comprising a cytoplasmic domain coding subsequence of a polynucleotide sequence selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a cytoplasmic domain, said cytoplasmic domain comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof; (c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1; and (d) a nucleotide sequence that encodes a cytoplasmic domain of a B7-1 polypeptide.

269. An isolated or recombinant polypeptide variant comprising an amino acid sequence that differs from the amino acid sequence of a primate B7-1, wherein the difference between the amino acid sequence of the variant and the amino acid sequence of the primate B7-1 comprises a different amino acid at position 65 other than alanine, wherein the position corresponds to the position in the amino acid sequence of human B7-1 of SEQ ID NO:278.

270. The isolated or recombinant polypeptide variant of claim 269, wherein the different amino acid is selected from the group of His, Arg, Lys, Pro, Phe, and Trp.

271. The isolated or recombinant polypeptide variant of claim 270, wherein the primate B7-1 is human B7-1 and the different amino acid is histidine.

272. The isolated or recombinant polypeptide variant of claim 271, wherein the variant has a CTLA-4/CD28 binding affinity ratio greater than the CTLA-41CD28 binding affinity ratio of human B7-1.

273. The isolated or recombinant polypeptide variant of claim 271, wherein the variant has an ability to induce a T cell proliferation response that is about equal to or less than the T cell proliferation response induced by human B7-1.

274. An isolated or recombinant polypeptide variant comprising an amino acid sequence that differs from the amino acid sequence of a primate B7-2, wherein the difference between the amino acid sequence of the variant and the amino acid sequence of the primate B7-2 comprises a different amino acid at position 56 of the B7-2 polypeptide sequence shown at GenBank Acc. No. AAA58389 or at position 50 of the B7-2 polypeptide sequence shown at GenBank Acc. No. AAA86473.

275. The isolated or recombinant polypeptide variant of claim 274, wherein the different amino acid is selected from the group of His, Arg, Lys, Pro, and/or Trp.

276. The isolated or recombinant polypeptide variant of claim 275, wherein the primate B7-2 is human B7-2 and the different amino acid is histidine.

277. The isolated or recombinant polypeptide variant of claim 276, wherein the variant has a CTLA-4/CD28 binding affinity ratio greater than the CTLA-4/CD28 binding affinity ratio of human B7-1 or B7-2.

278. The isolated or recombinant polypeptide variant of claim 276, wherein the variant has wherein the variant has an ability to induce a T cell proliferation response that is about equal to or less than the T cell proliferation response induced by human B7-1 or B7-2.

279. An isolated or recombinant polypeptide variant comprising an amino acid sequence that differs from the extracellular domain (ECD) amino acid sequence of a bovine B7-1, wherein the difference between the amino acid sequence of the variant and the ECD amino acid sequence of the bovine B7-1 comprises a different amino acid at one or more of the following amino acid residue positions: position 110, 124, 135, 192, 197, 199, 211, 213, 217, 218, 221, 225, 227, 231, 233, 235, 236, 237, 239, 240, 242, 243, and 244, wherein each position corresponds to the

position in the amino acid sequence of bovine B7-1 of SEQ ID NO:280.

280. The isolated or recombinant polypeptide variant of claim 279, wherein the difference between the amino acid sequence of the variant and the ECD amino acid sequence of the bovine B7-1 comprises at least one of: (a) a different amino acid at position 110, wherein the different amino acid is proline; (b) a different amino acid at position 124, wherein the different amino acid is valine; (c) a different amino acid at position 135, wherein the different amino acid is alanine; (d) a different amino acid at position 192, wherein the different amino acid is valine; (e) a different amino acid at position 197, wherein the different amino acid is glycine; (f) a different amino acid at position 199, wherein the different amino acid is glutamic acid; (g) a different amino acid at position 211, wherein the different amino acid is valine; (h) a different amino acid at position 213, wherein the different amino acid is asparagines; (i) a different amino acid at position 217, wherein the different amino acid is isoleucine; (j) a different amino acid at position 218, wherein the different amino acid is valine; (k) a different amino acid at position 221, wherein the different amino acid is isoleucine; (l) a different amino acid at position 225, wherein the different amino acid is glutamic acid; (m) a different amino acid at position 227, wherein the different amino acid is serine; (n) a different amino acid at position 231, wherein the different amino acid is isoleucine; (o) a different amino acid at position 233, wherein the different amino acid is proline; (p) a different amino acid at position 235, wherein the different amino acid is serine; (q) a different amino acid at position 236, wherein the different amino acid is lysine; (r) a different amino acid at position 237, wherein the different amino acid is proline; (s) a different amino acid at position 239, wherein the different amino acid is glutamine; (t) a different amino acid at position 240, wherein the different amino acid is glutamic acid; (u) a different amino acid at position 242, wherein the different amino acid is proline; (v) a different amino acid at position 243, wherein the different amino acid is isoleucine; and (w) a different amino acid at position 244, wherein the different amino acid is aspartic acid.

281. The isolated or recombinant polypeptide variant of claim 280, wherein the difference between the amino acid sequence of the variant and the ECD amino acid sequence of the bovine B7-1 further comprises at least one of: (1) a different amino acid at position 246, wherein the different amino acid is leucine; (2) a different amino acid at position 247, wherein the different amino acid is proline; (3) a different amino acid at position 248, wherein the different amino acid is phenylalanine; (4) a different amino acid at position 250, wherein the different amino acid is valine; and (5) a different amino acid at position 253, wherein the different amino acid is proline, wherein each position corresponds to the position in the amino acid sequence of bovine B7-1 of SEQ ID NO:280.

282. The isolated or recombinant polypeptide variant of claim 280, further comprising a **signal** peptide.

283. The isolated or recombinant polypeptide variant of claim 282, further comprising at least one of a transmembrane domain **signal** peptide.

284. A nucleic acid comprising a polynucleotide sequence that encodes an isolated or recombinant polypeptide of claim 269, 274, 279, 280, or 281, or a complementary nucleic acid sequence thereof.

285. An isolated or recombinant nucleic acid comprising a polynucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of the nucleic acid of claim 269, 274, 279, 280, or 281.

286. The isolated or recombinant polypeptide of claim 1 or 3, wherein the ECD amino acid sequence at least about amino acid residues 35 to 244 of any of SEQ ID NOS:48-68, 174-182, 184-221, 283-285, and 290-293.

287. The isolated or recombinant polypeptide of claim 1 or 3, which comprises a further amino acid sequence corresponding to at least one of a **signal** peptide, a transmembrane domain, or a cytoplasmic domain of a co-stimulatory polypeptide.

288. The isolated or recombinant polypeptide of claim 287, wherein the co-stimulatory polypeptide is a B7-1 polypeptide.

289. The isolated or recombinant polypeptide of claim 288, wherein the B7-1 polypeptide is a mammalian B7-1 polypeptide.

290. The isolated or recombinant polypeptide of claim 289, wherein the further amino acid sequence corresponding to the **signal** peptide sequence comprises at least about amino acid residues 1-34 of a polypeptide selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

291. The isolated or recombinant polypeptide of claim 287, wherein the further amino acid sequence corresponding to the transmembrane domain comprises at least about amino acid residues 35-244, 35-243, or 35-242, 35-255, 35-254, or 35-253 of a polypeptide selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

292. The isolated or recombinant polypeptide of claim 287, wherein the further amino acid sequence corresponding to the cytoplasmic domain comprises a cytoplasmic domain of a polypeptide selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

293. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an ECD amino acid sequence encoded by an ECD coding nucleotide sequence, the ECD coding nucleotide sequence comprising a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of the ECD coding nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142 or the nucleotide coding sequence that encodes the ECD of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

294. The isolated or recombinant polypeptide of claim 293, further comprising a **signal** peptide amino acid sequence encoded by a **signal** peptide coding nucleotide sequence, the **signal** peptide coding nucleotide sequence selected from the group of: (a) a nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide sequence encodes a **signal** peptide; (b) a nucleotide sequence that encodes the **signal** peptide of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

295. The isolated or recombinant polypeptide of claim 294, further comprising a transmembrane domain (TMD) amino acid sequence encoded by a TMD nucleotide sequence selected from the group of: (a) a nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide sequence encodes a TMD polypeptide; (b) a nucleotide sequence that encodes the TMD of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

296. The isolated or recombinant polypeptide of claim 295, further comprising a cytoplasmic domain (CD) amino acid sequence encoded by a CD nucleotide sequence selected from the group of: (a) a nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide sequence encodes a CD polypeptide; (b) a nucleotide sequence that encodes the CD of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

297. The isolated or recombinant nucleic acid of claim 43, wherein the polynucleotide sequence of (d) comprises encodes a fragment of (a) or (b), wherein the fragment encodes an extracellular domain polypeptide having a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

298. The isolated or recombinant polypeptide of claim 96, further comprising a **signal** peptide amino acid sequence encoded by a **signal** peptide coding nucleotide sequence, the **signal** peptide coding nucleotide sequence selected from the group of: (a) a nucleotide sequence comprising a nucleotide fragment of a polynucleotide sequence selected from any of the group of SEQ ID NOS:22-45 and 143-173, wherein said nucleotide fragment encodes a **signal** peptide; (b) a nucleotide sequence that encodes the **signal** peptide of a polypeptide selected from any of the group of SEQ ID NOS:69-92, 222-252, and 286-289; and

(c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

299. The isolated or recombinant polypeptide of claim 298, further comprising a transmembrane domain (TMD) amino acid sequence encoded by a TMD nucleotide sequence selected from the group of: (a) a nucleotide sequence of a polynucleotide sequence selected from any of the group of SEQ ID NOS:22-45 and 143-173, wherein said nucleotide sequence encodes a TMD polypeptide; (b) a nucleotide sequence that encodes the TMD of a polypeptide selected from any of the group of SEQ ID NOS:69-92, 222-252, and 286-289; and (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

300. The isolated or recombinant polypeptide of claim 299, further comprising a cytoplasmic domain (CD) amino acid sequence encoded by a CD nucleotide sequence selected from the group of: (a) a nucleotide sequence of a polynucleotide sequence selected from any of the group of SEQ ID NOS:22-45 and 143-173, wherein said nucleotide sequence encodes a CD polypeptide; (b) a nucleotide sequence that encodes the CD of a polypeptide selected from any of the group of SEQ ID NOS:69-92, 222-252, and 286-289, and 290-293; and (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

301. An isolated or recombinant polypeptide comprising an amino acid sequence of at least an extracellular domain, wherein said extracellular domain amino acid sequence has at least about 75% amino acid sequence identity to an extracellular domain amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, and is not a naturally-occurring extracellular domain amino acid sequence, and wherein said polypeptide has an ability to induce T cell proliferation or T cell activation response that is equal to or greater than that of human B7-1.

302. The isolated or recombinant polypeptide of claim 1 or 301, wherein said polypeptide comprises more than one of the extracellular domain.

303. The isolated or recombinant polypeptide of claim 302, wherein said polypeptide comprises a multimer of the extracellular domain.

304. The isolated or recombinant polypeptide of claim 302, wherein said polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.

305. The isolated or recombinant polypeptide of claim 304, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

306. An isolated or recombinant polypeptide comprising an amino acid sequence of an extracellular domain, wherein said extracellular domain (ECD) amino acid sequence has at least about 75% amino acid sequence identity to an extracellular domain amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, and is not a naturally-occurring extracellular domain amino acid sequence, and wherein said polypeptide, in the presence of a population of activated T cells, has an ability to induce a T cell proliferation or T cell activation response that is less than that induced by a human B7-1 ECD amino acid sequence in the presence of a population of activated T cells.

307. The isolated or recombinant polypeptide of claim 1 or 306, wherein said polypeptide comprises a soluble ECD monomer.

308. The isolated or recombinant polypeptide of claim 307, wherein said soluble ECD monomer further comprises an Ig polypeptide.

309. A soluble isolated or recombinant multimeric polypeptide comprising at least two polypeptides of claim 1 or 306.

310. The polypeptide of claim 309, wherein each of said at least two polypeptides further comprises at least one additional amino acid sequence which comprises an Ig polypeptide.

311. A nucleic acid comprising a polynucleotide sequence that encodes an isolated or recombinant polypeptide of claim 259, 269, 271, 275, 276,

279, 280, 301, or 306, or a complementary polynucleotide sequence thereof.

312. A vector comprising at least one nucleic acid which comprises a polynucleotide sequence that encodes an isolated or recombinant polypeptide of claim 259, 269, 271, 275, 276, 279, 280, 301, or 306, or a complementary polynucleotide sequence thereof.

313. A composition comprising a nucleic acid of claim 311 and a carrier.

314. A composition comprising a polypeptide of claim 259, 269, 271, 275, 276, 279, 280, 301, or 306 and a carrier.

315. A recombinant nucleic acid molecule comprising a polynucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of the nucleic acid of claim 311 or 312.

316. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has an CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

317. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide comprises a soluble polypeptide having an ability in the presence of a population of activated T cells to induce a T cell proliferation response that is less than the T cell proliferation response induced by a soluble human B7-1 polypeptide in the presence of a population of activated T cells.

318. The isolated or recombinant polypeptide of claim 171, wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, and/or an ability to induce a T-cell proliferation or T-cell activation response about equal to or greater than that of hB7-1.

319. The isolated or recombinant nucleic acid of claim 170, wherein said nucleic acid encodes a polypeptide that has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, and/or an ability to induce a T-cell proliferation or T-cell activation response about equal to or less than that of hB7-1.

320. The isolated or recombinant polypeptide of claim 1, 5, 6, or 7, wherein the polypeptide is bound to a cell membrane and has an ability to induce T-cell proliferation or T-cell activation or both.

L6 ANSWER 6 OF 18 USPATFULL on STN
2003:123208 Non-A, non-B, non-C, non-D, non-E hepatitis reagents and methods for their use.
Simons, John N., Grayslake, IL, United States
Pilot-Matias, Tami J., Green Oaks, IL, United States
Dawson, George J., Libertyville, IL, United States
Schlauder, George G., Skokie, IL, United States
Desai, Suresh M., Libertyville, IL, United States
Leary, Thomas P., Kenosha, WI, United States
Muerhoff, Anthony Scott, Kenosha, WI, United States
Erker, James Carl, Hainesville, IL, United States
Buijk, Sheri L., Round Lake, IL, United States
Mushahwar, Isa K., Grayslake, IL, United States
Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)
US 6558898 B1 20030506
APPLICATION: US 1995-488446 19950607 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Hepatitis GB Virus (HGBV) nucleic acid and amino acid sequences useful for a variety of diagnostic and therapeutic applications, kits for using the HGBV nucleic acid or amino acid sequences, HGBV immunogenic particles, and antibodies which specifically bind to HGBV. Also provided are methods for producing antibodies, polyclonal or monoclonal, from the HGBV nucleic acid or amino acid sequences.
CLM What is claimed is:
1. A purified antibody which specifically binds to at least one hepatitis GB virus (HGBV) epitope of an HGBV antigen, wherein said epitope comprises at least 5 amino acids and wherein said HGBV antigen (a) is encoded by a positive stranded RNA viral genome wherein said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 387 and (b) is not immunoreactive with an antibody that specifically binds to HGBV-B or HGBV-C.

2. The antibody of claim 1 wherein said antibody is polyclonal.

3. The antibody of claim 1 wherein said antibody is monoclonal.

4. The antibody of claim 1 attached to a **signal** generating compound.

5. An assay kit useful for determining the presence of hepatitis GB virus (HGBV) antigen or antibody in a test sample comprising a container containing a polypeptide possessing at least one HGBV antigen, wherein said HGBV antigen (a) is encoded by a positive stranded RNA viral genome wherein said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 387 and (b) is not immunoreactive with an antibody that specifically binds to HGBV-B or HGBV-C.

6. A method for detecting hepatitis GB virus (HGBV) antibodies in a test sample suspected of containing said antibodies, comprising: (a) contacting the test sample with a polypeptide, wherein said polypeptide (i) contains at least one HGBV epitope comprising at least 5 amino acids, (ii) is encoded by a positive stranded RNA viral genome wherein said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 387, and (iii) is not immunoreactive with an antibody that specifically binds to HGBV-B or HGBV-C, for a time and under conditions sufficient to allow antigen/antibody complexes to form; (b) detecting said complexes which contain the HGBV antibodies.

7. The method of claim 6 wherein said polypeptide is produced by recombinant technology or synthetic means.

8. The method of claim 6, wherein step (b) further comprises contacting said complexes with an indicator reagent prior to detecting said complexes.

9. The method of claim 8, wherein said indicator reagent comprises a **signal** generating compound.

10. A method for detecting hepatitis GB virus (HGBV) antigen in a test sample suspected of containing HGBV comprising: (a) contacting the test sample with an antibody or fragment thereof which specifically binds to at least one epitope of HGBV antigen, wherein said epitope comprises at least 5 amino acids, for a time and under conditions sufficient to allow for the formation of antibody/antigen complexes; (b) detecting said complexes containing the HGBV antigen in the test sample, wherein said HGBV antigen (i) is encoded by a positive stranded RNA viral genome wherein said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 387, and (ii) is not immunoreactive with an antibody that specifically binds to HGBV-B or HGBV-C.

11. The method of claim 10 wherein step (b) further comprises contacting said complexes with an indicator reagent and incubating said complexes and said indicator reagent prior to detecting said complexes.

12. The method of claim 11, wherein said indicator reagent comprises a **signal** generating compound.

13. A purified antibody which specifically binds to at least one hepatitis GB virus (HGBV) epitope of an HGBV antigen, wherein said epitope comprises at least 5 amino acids and wherein said HGBV antigen (a) is encoded by a positive stranded RNA viral genome, wherein said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 394 and (b) is not immunoreactive with an antibody that specifically binds to HGBV-A or HGBV-C.

14. An assay kit useful for determining the presence of hepatitis GB virus (HGBV) antigen or antibody in a test sample comprising a container containing a polypeptide possessing at least one HGBV antigen, wherein said HGBV antigen (a) is encoded by a positive stranded RNA viral genome wherein said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 394 and (b) is not immunoreactive with an antibody that specifically binds to HGBV-A or HGBV-C.

15. A method for detecting hepatitis GB virus (HGBV) antibodies in a test sample suspected of containing said antibodies, comprising: (a) contacting the test sample with a polypeptide wherein said polypeptide (i) contains at least one HGBV epitope comprising at least 5 amino acids, (ii) is encoded by a positive stranded RNA viral genome, wherein

said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 394, and (iii) is not immunoreactive with an antibody that specifically binds to HGBV-A or HGBV-C, for a time and under conditions sufficient to allow antigen/antibody complexes to form; (b) detecting said complexes which contain the HGBV antibodies.

16. A method for detecting hepatitis GB virus (HGBV) antigen in a test sample suspected of containing HGBV comprising: (a) contacting the test sample with an antibody or fragment thereof which specifically binds to at least one epitope of HGBV antigen, wherein said epitope comprises at least 5 amino acids, for a time and under conditions sufficient to allow for the formation of antibody/antigen complexes; (b) detecting said complexes containing the HGBV antigen in the test sample, wherein said HGBV antigen (i) is encoded by a positive stranded RNA viral genome, wherein said viral genome encodes a polyprotein, wherein said polyprotein comprises SEQUENCE I.D. NO. 394, and (ii) is not immunoreactive with an antibody that specifically binds to HGBV-A or HGBV-C.

L6 ANSWER 7 OF 18 USPATFULL on STN
2002:297432 Non-stochastic generation of genetic vaccines.
Short, Jay M., Rancho Santa Fe, CA, United States
Diversa Corporation, San Diego, CA, United States (U.S. corporation)
US 6479258 B1 20021112
APPLICATION: US 2000-495052 20000131 (9)
PRIORITY: US 1995-8311P 19951207 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods of obtaining vaccines by use of non-stochastic methods of directed evolution (DirectEvolution.TM.). These methods include non-stochastic polynucleotide site-satuaration mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). Through use of the claimed methods, vectors can be obtained which exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like.

CLM What is claimed is:

1. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, wherein optimization is achieved by at least one directed evolution method in any combination, permutation and iterative manner.

2. The method of claim 1, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

3. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization,or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: screening a library Qf non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created.

4. The method of claim 3, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

5. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune

response induced by a vector; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created; and whereby optimization is achieved using one or more directed evolution methods in any combination, permutation, and iterative manner.

6. The method of claim 5, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

7. The method of any claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide is incorporated into a vector.

8. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide, or a polypeptide encoded by the optimized non-stochastically generated polynucleotide, is administered in conjunction with a vector.

9. The method of any of claims 1, 3 or 5, wherein the library of non-stochastically generated progeny polynucleotides is created by a process selected from gene reassembly or oligonucleotide-directed saturation mutagenesis, and any combination, permutation and iterative manner.

10. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response is obtained by: a) non-stochastically reassembling at least two parental template polynucleotide, each of which is, or encodes a molecule that is, involved in modulating an immune response; wherein the first and second parental templates differ from each other in two or more nucleotides, to produce a library of non-stochastically generated polynucleotides; and b) screening the library to identity at least one optimized non-stochastically generated polynucleotide that exhibits, either by itself or through the encoded molecule, an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.

11. The method of claim 10, wherein the method further comprises the steps of: c) subjecting an optimized non-stochastically generated polynucleotide to a further round of non-stochastic reassembly with at least one additional polynucleotide, which is the same or different from the first and second polynucleotides, to produce a further library of recombinant polynucleotides; d) screening the library produced in c) to identify at least one further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created; and e) optionally repeating c) and d) as necessary, until a desirable further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response than a form of the nucleic acid from which the library was created.

12. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that interacts with a cellular receptor involved in mediating an immune response; wherein the polypeptide acts as an agonist or antagonist of the receptor.

13. The method of claim 12, wherein the cellular receptor is a macrophage scavenger receptor.

14. The method of claim 12, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine receptor.

15. The method of claim 14, wherein the chemokine receptor is CCR5 or CCR6.

16. The method of claim 12, wherein the polypeptide mimics the activity of a natural ligand for the receptor but does not induce immune reactivity to said natural ligand.

17. The method of claim 12, wherein the library is screened by: i) expressing the non-stochastically generated progeny polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of cells that display

the receptor; and iii) identifying cells that exhibit a modulation of an immune response mediated by the receptor.

18. The method of claim 17, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.

19. The method of claim 18, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.

20. The method of claim 12, which method further comprises introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a mammal, wherein the peptide or polypeptide is expressed and acts as an agonist or antagonist of the receptor.

21. The method of claim 12, which method further comprises producing the polypeptide encoded by the optimized non-stochastically generated polynucleotide and introducing the polypeptide into a mammal in conjunction with a genetic vaccine vector.

22. The method of claim 12, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.

23. The method of claim 22, wherein the optimized non-stochastically generated polypeptide is introduced into a nucleotide sequence that encodes an M-loop of an HBsAg polypeptide.

24. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.

25. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that inhibits an allergic reaction.

26. The method of claim 25, wherein the polypeptide is selected from the group consisting of interferon-α, interferon-γ, IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.

27. The method of 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.

28. The method of claim 27, wherein the antagonist of IL-10 is soluble or defective IL-10 receptor or IL-20/MDA-7.

29. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

30. The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4.

31. The method of claim 29, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).

32. The method of claim 29, wherein the co-stimulator is a cytokine.

33. The method of claim 32, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF-α, IFN-α, IFN-γ, and IL-20 (MDA-7).

34. The method of 33, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.

35. The method of claim 34, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.

36. The method of 33, wherein the cytokine is interleukin-12 and the screening is performed by: growing mammalian cells which contain the genetic vaccine vector in a culture medium; and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.

37. The method of 33, wherein the cytokine is interferon-α and the screening is performed by: i) expressing the non-stochastically generated polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of B cells; and iii) identifying phage library members that are capable of inhibiting proliferation of the B cells.

38. The method of claim 33, wherein the immune response of interest is differentiation of T cells to T_{H1} cells and the screening is performed by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon-γ.

39. The method of claim 32, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide, and the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a mammal and determining whether an immune response is induced against the cytokine.

40. The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the cell is tested for ability to costimulate an immune response.

41. The method of any of claims 1, 3, or 5, wherein the optimized recombinant polynucleotide encodes a cytokine antagonist.

42. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor and a transmembrane cytokine receptor having a defective **signal sequence**.

43. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of ΔIL-1 OR and ΔIL-4R.

44. The method of any of claims 1, 3, or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H1} immune response.

45. The method of any of claims 1, 3, or 5 wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H2} immune response.

46. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having decreased antigenicity with respect to at least one host recipient of said molecule.

47. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is an increase in a desirable modulatory effect on an immune response; whereby application of the method can. be used to generate a molecule having an increased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having increased antigenicity with respect to at least one host recipient of said molecule.

48. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response as well as an increase in a second desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule as well as a an increased ability to elicit a second immune response from a second host recipient of said molecule; whereby the first and the second recipient hosts can be the same or different; whereby each of the first and the second recipient hosts can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having both a first decreased antigenicity with respect to at least one host recipient of said

molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.

49. The method of claim 48, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector; whereby a module is exemplified by the following modules, as well as by a fragment derivative or analog thereof: an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin or replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker, and enhancer, a promoter, and operator, and an intron.

50. The method of any of claims 1, 3, or 5, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; whereby application of the method can be used to generate a molecule having an increased stability ex vivo, thus, for example, increasing shelf-life and/or ease of storage and/or length of time before expiration of activity upon storage; and whereby application of the method can also be used to generate a molecule having an increased stability in vivo upon administration to a host recipient, thus, for example, increasing resistance to digestive acids and/or increasing stability in the circulation and/or any other method of elimination or destruction by the host recipient.

51. The method of any of claims 1, 3, or 5, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in a human host recipient as compared with prior to optimization; whereby application of the method can thus be used to generate an optimized genetic vaccine for human recipients.

52. The method of any of claims 1, 3, or 5, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in an animal host recipient as compared with prior to optimization; whereby application of the method can thus be used to generate an optimized genetic vaccine for animal recipients, including animals that are farmed or raised by man, animals that are not fanned or raised by man, domesticated animals, and non-domesticated animals.

53. A method for obtaining an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, the method comprising: a) creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly; whereby application of the method can thus be used to generate an optimized molecule for human recipients &/or animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

54. The method of claim 53, wherein the screening involves: i) introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen to form a library of vectors; introducing the library of vectors into mammalian cells; and ii) identifying mammalian cells that exhibit increased or decreased immunogenicity to the antigen.

55. The method of claim 53, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.

56. The method of claim 53, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.

57. The method of claim 56, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.

58. The method of claim 53, wherein the accessory molecule comprises an immunogenic agonist sequence.

59. A method for obtaining an immunomodulatory polynucleotide that has, an optimized expression in a recombinant expression host, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner; whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

60. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created.

61. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

62. The method of any of claims 59-61, wherein the recombinant expression host is a prokaryote.

63. The method of any of claims 59-61, wherein the recombinant expression host is a eukaryote.

64. The method of claim 63, wherein the recombinant expression host is a plant.

65. The method of claim 64, wherein the recombinant expression host is a monocot.

66. The method of claim 64, wherein the recombinant expression host is a dicot.

67. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "gene site saturation mutagenesis" as described herein.

68. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "synthetic ligation polynucleotide reassembly" as described herein.

69. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a

parental polynucleotide set is comprised of subjecting the parental polynucleotide set to both "gene site saturation mutagenesis" as described herein, and to "synthetic ligation polynucleotide reassembly" as described herein.

70. The method of claim 1, wherein the directed evolution method is synthetic ligation reassembly.

71. The method of claim 1, wherein the directed evolution method is gene site saturated mutagenesis.

72. The method of claim 1, wherein the directed evolution method is non-stochastic ligation reassembly.

73. The method of claim 1, wherein the directed evolution method is exonuclease-mediated reassembly.

74. The method of claim 1, wherein the directed evolution method is end selection.

75. The method of claim 1, wherein the directed evolution method is shuffling.

76. The method of claim 1, wherein the immunomodulatory polynucleofide encodes a cancer antigen.

77. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a bacterial antigen.

78. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a viral antigen.

79. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a parasite antigen.

80. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a self-antigen.

81. The method of claim 1, wherein the immune response is a humoral immune response.

82. The method of claim 1, wherein the immune response is a cellular immune response.

83. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a cytokine.

84. The method of claim 83, wherein the cytokine is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF-α, IFN-α, IFN-γ, or IL-20 (MDA-7).

85. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined in vitro.

86. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined in vivo.

L6 ANSWER 8 OF 18 USPATFULL on STN
2002:157109 Novel chimeric promoters.
Punnonen, Juha, Belmont, CA, UNITED STATES
Wright, Anne, Woodside, CA, UNITED STATES
Semyonov, Andrey, San Francisco, CA, UNITED STATES
US 2002081708 A1 20020627
APPLICATION: US 2001-886942 A1 20010621 (9)
PRIORITY: US 2000-213829P 20000623 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides novel chimeric promoter/enhancers. The chimeric promoter/enhancers are particularly suitable for directing gene expression in mammalian cells.
CLM What is claimed is:
1. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from the group consisting of: (a) a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO:18 or a complementary polynucleotide sequence thereof; (b) a polynucleotide sequence that has

at least about 97% sequence identity to at least one sequence from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18 or a complementary polynucleotide sequence thereof; (c) a polynucleotide sequence that has at least about 80% sequence identity to at least one sequence from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18, or a complementary polynucleotide sequence thereof, wherein said polynucleotide sequence promotes expression of an operably linked transgene at a level that is greater than the level of expression of the same transgene when operably linked to a human CMV promoter polynucleotide sequence; and (d) a polynucleotide sequence comprising a fragment of (a), (b), or (c), wherein said fragment promotes expression of an operably linked transgene at a level that is greater than the level of expression of the same transgene when operably linked to a human CMV promoter polynucleotide sequence.

2. The nucleic acid of claim 1, comprising a polynucleotide sequence of (b), wherein said polynucleotide sequence promotes expression of an operably linked transgene at a level that is equal to or greater than the level of expression of the same transgene when operably linked to a human CMV promoter polynucleotide sequence.

3. The nucleic acid of claim 1, wherein the human CMV promoter polynucleotide sequence is a Towne or AD169 human CMV promoter polynucleotide sequence.

4. The nucleic acid of claim 1, comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18 or a complementary polynucleotide sequence thereof.

5. The nucleic acid of claim 1, comprising a polynucleotide sequence that has at least about 97% sequence identity to at least one sequence from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18 or a complementary polynucleotide sequence thereof.

6. The nucleic acid of claim 1, comprising a polynucleotide sequence that has at least about 98% sequence identity to at least one sequence from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18 or a complementary polynucleotide sequence thereof.

7. The nucleic acid of claim 1, comprising a polynucleotide sequence that has at least about 99% sequence identity to at least one sequence from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18 or a complementary polynucleotide sequence thereof.

8. The nucleic acid of claim 1, comprising a polynucleotide sequence that has at least about 80% sequence identity to at least one sequence from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18, or a complementary polynucleotide sequence thereof, wherein said polynucleotide sequence promotes expression of an operably linked transgene at a level that is greater than the level of expression of the same transgene when operably linked to a human CMV promoter polynucleotide sequence.

9. The nucleic acid of claim 1, comprising a polynucleotide sequence comprising a fragment of claim 1 (a), (b), or (c), wherein said fragment promotes expression of an operably linked transgene at a level that is greater than the level of expression of the same transgene when operably linked to a human CMV promoter polynucleotide sequence.

10. An isolated or recombinant nucleic acid comprising a fragment of one sequence from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 18 or a fragment of a complementary polynucleotide sequence thereof, wherein the fragment comprises a unique subsequence.

11. The nucleic acid of claim 10, wherein the fragment promotes the expression of a transgene to which the fragment is operably linked.

12. An isolated or recombinant nucleic acid comprising a polynucleotide sequence that hybridizes under highly stringent conditions over substantially the entire length of a polynucleotide sequence of claim 1 (a), (b), (c), or (d).

13. The nucleic acid of claim 12, wherein the highly stringent conditions are selected such that a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 18 hybridizes to its perfect complement with at least a 5-fold higher **signal** to noise ratio than for hybridization of the perfect complement to a control nucleic acid comprising a human CMV promoter polynucleotide sequence.

14. The nucleic acid of claim 1, comprising a polynucleotide sequence that promotes the expression of an operably linked transgene at a level that differs from the expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

15. The nucleic acid of claim 14, wherein the transgene is luciferin luciferase, and transgene expression level is determined in an in vitro luciferase assay.

16. The nucleic acid of claim 14, wherein the transgene is β-galactosidase, the transgene is expressed in vivo, and transgene expression level is determined by measuring the serum titer of anti-β-galactosidase antibodies.

17. The nucleic acid of claim 14, wherein the polynucleotide sequence promotes the expression of an operably linked transgene at a level that is higher than the highest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

18. The nucleic acid of claim 17, wherein polynucleotide sequence promotes the expression of an operably linked transgene at a level that is 2-fold higher than the highest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

19. The nucleic acid of claim 14, wherein the polynucleotide sequence promotes the expression of an operably linked transgene at a level that is lower than the lowest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

20. The nucleic acid of claim 19, wherein polynucleotide sequence promotes the expression of an operably linked transgene at a level that is 2-fold lower than the lowest expression level of the same transgene when operably linked to a nucleic acid sequence corresponding to a human CMV promoter polynucleotide sequence.

21. The nucleic acid of claim 1, wherein the nucleic acid comprises a deletion of one or more nucleotides in a region corresponding to about nucleotides 830-835 or 841-844 of the consensus sequence shown in FIG. 8.

22. The nucleic acid of claim 21, wherein the nucleic acid comprises a deletion of nucleotides corresponding to about nucleotides 830-835 or 841-844 of the consensus sequence.

23. The nucleic acid of claim 22, wherein the nucleic acid comprises a deletion of nucleotides corresponding to about nucleotides 830-835 and 841-844 of the consensus sequence.

24. The nucleic acid of claim 1, wherein the nucleic acid comprises a Rhesus monkey CMV promoter polynucleotide sequence at about nucleotide positions 817-863, numbered according to the consensus sequence shown in FIG. 8.

25. The nucleic acid of claim 1, wherein the nucleic acid comprises a polynucleotide sequence selected from GACGCCGGAGG and GACGTCGGAG.

26. The nucleic acid of claim 1, wherein the nucleic acid comprises an insertion of a nucleotide, as compared to the human Towne CMV promoter sequence, after nucleotide position 853, numbered according to the consensus sequence shown in FIG. 8.

27. The nucleic acid of claim 1, wherein the nucleic acid comprises a deletion of one or more nucleotides in a region corresponding to about nucleotides 684-735 of the consensus sequence shown in FIG. 8.

28. The nucleic acid of claim 27, wherein the nucleic acid comprises a deletion of any nucleotides corresponding to about nucleotides 684-735 of the consensus sequence.

29. The nucleic acid of claim 1, wherein the nucleic acid comprises the polynucleotide sequence AATGGGCGGTC.

30. The nucleic acid of claim 1, wherein the nucleic acid does not comprise CMV promoter nucleic acid residues beyond about nucleotide residue 909, numbered according to the consensus sequence shown in FIG.

8.

31. The nucleic acid of claim 1, wherein the nucleic acid comprises a polynucleotide sequence comprising nucleic acid residue 1 to about nucleotide residue 930, numbered according to the consensus sequence shown in FIG. 8.

32. The nucleic acid of claim 31, wherein the nucleic acid does not comprise CMV promoter nucleic acid residues beyond about nucleotide residue 930, numbered according to the consensus sequence.

33. The nucleic acid of claim 1, wherein the nucleic acid comprises a polynucleotide sequence comprising nucleic acid residue 1 to nucleotide residue 932, numbered according to the consensus sequence shown in FIG. 8.

34. The nucleic acid of claim 33, wherein the nucleic acid does not comprise CMV nucleotide residues beyond nucleotide residue 932, numbered according to the consensus sequence shown in FIG. 8.

35. The nucleic acid of claim 1, wherein the nucleic acid comprises a deletion of one or more nucleotides in a region corresponding to about nucleotide residues 319-512 of the consensus sequence shown in FIG. 8.

36. The nucleic acid of claim 35, wherein the nucleic acid comprises a deletion of nucleotides corresponding to about nucleotide residues 319-512 of the consensus sequence.

37. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:21 or a complementary polynucleotide sequence thereof.

38. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:8 (6A8) or a complementary polynucleotide sequence thereof.

39. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO: 11 (6F6) or a complementary polynucleotide sequence thereof.

40. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:6 (3C9) or a complementary polynucleotide sequence thereof.

41. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:9 (6B2) or a complementary polynucleotide sequence thereof.

42. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:2 (11E2) or a complementary polynucleotide sequence thereof.

43. The nucleic acid of claim 1, wherein the polynucleotide sequence comprises SEQ ID NO:3 (12C9) or a complementary polynucleotide sequence thereof.

44. The nucleic acid of claims 1, 10 or 12, wherein the polynucleotide sequence is operably linked to a transgene to form an expression cassette.

45. The nucleic acid of claim 44, wherein the transgene is a viral gene.

46. The nucleic acid of claim 44, wherein the transgene encodes a polypeptide selected from the group consisting of an immunogen, an immunomodulatory molecule, an antigen, an adjuvant, an allergen, an antibody, a bacterial toxin, a cytokine, a cytokine receptor, and a co-stimulatory molecule.

47. The nucleic acid of claim 46, wherein the transgene encodes an antigen selected from the group consisting of a cancer antigen, a hepatitis B surface antigen, a hepatitis A antigen, and a hepatitis C antigen.

48. The nucleic acid of claim 46, wherein the transgene encodes a co-stimulatory molecule comprising a polypeptide that binds to a CD28 or CTLA-4 receptor.

49. A composition produced by the cleaving of one or more nucleic acids of claims 1, 10, or 12, wherein the cleaving comprises mechanical,

chemical, or enzymatic cleavage.

50. The composition of claim 49, wherein the cleaving comprises enzymatic cleavage with a restriction endonuclease, an RNAse or a DNAse.

51. A composition produced by a process comprising incubating one or more nucleic acids of claims 1, 10, or 12 in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase.

52. The composition of claim 51, wherein the nucleic acid polymerase is a thermostable polymerase.

53. A method of producing a modified or recombinant nucleic acid comprising mutating or recombining a nucleic acid of claims 1, 10, or 12.

54. The method of claim 53, comprising recursively recombining the nucleic acid with one or more additional nucleic acids.

55. The method of claim 54, wherein the one or more additional nucleic acids promote the expression of an operably linked transgene.

56. The method of claim 54, wherein the recursive recombination is performed in vitro.

57. The method of claim 54, wherein the recursive recombination is performed in vivo.

58. The method of claim 54, wherein the recursive recombination produces at least one library of recombinant nucleic acids, which library comprises at least one recombinant nucleic acid that promotes the expression of an operably linked transgene.

59. The method of claim 53 additionally comprising assaying the modified or recombinant nucleic acid produced by the method for the ability to promote the expression of an operably linked transgene.

60. A nucleic acid library produced by the method of claim 53.

61. A nucleic acid library comprising two or more nucleic acids of claims 1, 10, or 12.

62. A vector comprising at least one nucleic acid of claims 1, 10, 12 or 44.

63. The vector of claim 62, wherein the vector is an expression vector.

64. The vector of claim 62, wherein the vector is selected from a plasmid, a cosmid, a phage, a virus or fragment thereof, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC).

65. A cell comprising the nucleic acid of claims 1, 10, or 12 or the vector of claim 62.

66. The cell of claim 65, wherein the cell comprises a human cell.

67. A population of cells comprising the library of claims 60 or 61.

68. A composition comprising the nucleic acid of claims 1, 10, or 12 or the vector of claim 62 and a carrier.

69. The composition of claim 68, wherein the excipient is a pharmaceutically acceptable carrier.

70. The composition of claim 48, wherein the nucleic acid or vector is present in the composition in an amount sufficient to introduce the nucleic acid or vector into cells of a subject, when the composition is administered to the subject.

71. A composition comprising the nucleic acid of claims 1, 10, or 12 or the vector of claim 62 in an amount sufficient to introduce the nucleic acid or vector into cells of a subject, when the composition is administered to the subject.

72. The composition of claims 70 or 71, wherein the amount is sufficient to introduce the nucleic acid or vector into cells of a subject, when the composition is administered to the subject by a route selected from the group consisting of topical administration, injection, implantation, oral administration, buccal, vaginal administration, rectal

administration, and inhalation.

73. The composition of claim 75, wherein the composition is administered to the subject by a route selected from the group consisting of intradermal, subdermal, subcutaneous, intramuscular, intravenous, intraperitoneal, and intrathecal.

74. A method of producing a polypeptide, the method comprising: (a) providing a population of cells comprising a nucleic acid of claims 1, 10, or 12 operably linked to a transgene encoding a polypeptide; and (b) expressing the polypeptide in at least the subset of the population of cells or progeny thereof.

75. The method of claim 74, wherein the population of cells is provided by introducing the nucleic acid operably linked to the transgene into the population of cells.

76. The method of claim 74, further comprising isolating the polypeptide from the cells.

77. The method of claim 74, wherein the cells are in culture.

78. The method of claim 77, comprising expressing the polypeptide by culturing the population or subset of the population of cells or progeny thereof in a nutrient medium under conditions in which the nucleic acid promotes expression of the polypeptide.

79. The method of claim 78, further comprising isolating or recovering the polypeptide from the cells or from the nutrient medium.

80. The method of claim 74, wherein the cells comprise mammalian cells selected from fertilized oocytes, embryonic stem cells, or pluripotent stem cells, the method further comprising generating a transgenic mammal expressing the polypeptide.

81. The method of claim 80, further comprising recovering the polypeptide from the transgenic mammal or a byproduct of the transgenic mammal.

82. The method of claim 74, wherein the cells are in vivo in a subject.

83. The method of claim 82, wherein the nucleic acid is introduced into cells in culture, and the cells are subsequently introduced into the subject.

84. The method of claim 82, wherein the nucleic acid is introduced into the cells of the subject by administering the nucleic acid directly to the subject.

85. The method of claim 84, wherein the nucleic acid is administered to the subject by a route selected from the group consisting of topical administration, injection, implantation, oral administration, vaginal administration, rectal administration, and inhalation.

86. The method of claim 85, wherein the nucleic acid is administered to the subject by a route selected from the group consisting of intradermal, subdermal, subcutaneous, intramuscular, intravenous, intraperitoneal, and intrathecal.

87. The method of claim 84, wherein the nucleic acid is administered to the subject by topical administration, injection, or using a gene gun.

88. The method of claim 82, wherein the subject is a human.

89. The method of claim 82, wherein the polypeptide is expressed in an amount sufficient to produce a desired effect in the subject.

90. The method of claim 89, wherein the desired effect comprises an immunogenic effect, a prophylactic effect, or a therapeutic effect.

91. A nucleic acid of claims 1, 10, or 12 for use in producing an immunogenic effect, a prophylactic effect, or a therapeutic effect in a subject.

92. The nucleic acid of claim 91, wherein the subject is a human.

93. A kit comprising a nucleic acid of claims 1, 10, 12, or 44.

94. A kit comprising a vector of claims 62 or 63.

95. A database comprising one or more character strings corresponding to a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 18 or a complementary polynucleotide sequence thereof.

96. A database comprising one or more character strings corresponding to a unique subsequence of a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 18 or a uniques subsequence of a complementary polynucleotide sequence thereof.

97. The database of claims 95 or 96, wherein the one or more character strings is recorded in a computer-readable medium.

98. A method for manipulating a sequence record in a computer system, the method comprising: (a) reading a character string corresponding to a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 18, or a complementary polynucleotide sequence thereof; (b) performing an operation on the character string; and (c) returning a result of the operation.

99. A method for manipulating a sequence record in a computer system, the method comprising: (a) reading a character string corresponding to a unique subsequence of a polynucleotide sequence selected from SEQ ID NO:1 to SEQ ID NO:18 or a uniques subsequence of a complementary polynucleotide sequence thereof; (b) performing an operation on the character string; and (c) returning a result of the operation.

100. The method of claims 98 or 99, wherein the user selects the character string from a database or inputs the character string into the computer system.

101. The method of claims 98 or 99, comprising performing one or more operations selected from among: a local sequence comparison, a sequence alignment, a sequence identity or similarity search, a sequence identity or similarity determination, a nucleic acid motif determination, a hypothetical translation, a determination of a restriction map, a sequence recombination, or a BLAST determination.

102. The method of claim 101, comprising aligning the selected character string with one or more additional character strings corresponding to a polynucleotide sequence.

103. The method of claim 101, wherein the operation comprises transmitting the character string to a device capable of producing a nucleic acid comprising the polynucleotide sequence corresponding to the character string.

L6 ANSWER 9 OF 18 USPATFULL on STN
2002:95363 Immunological combination compositions and methods.
Becker, Robert S., Henryville, PA, United States
Huebner, Robert C., Stroudsburg, PA, United States
Gray, Maryann, Bartonsville, PA, United States
Biscardi, Karen S., South Sterling, PA, United States
Erdile, Lorne F., Tassin la Demi Lune, FRANCE
Guy, Bruno, Lyons, FRANCE
Connaught Laboratories, Inc., Swiftwater, PA, United States (U.S. corporation)
US 6379675 B1 20020430
APPLICATION: US 1996-588621 19960119 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Immunological compositions and methods for making and using them. The compositions contain at least one antigen and at least one lipoprotein and optionally an adjuvant. The lipoprotein can itself be antigenic or immunogenic. The antigen can be influenza HA and the lipoprotein a recombinantly expressed product having an OspA leader for lipidation and PspA for the protein portion. The antigen can be OspC and the lipoprotein OspA. The components of the composition are co-administered. A potentiated immunological response is obtained by the compositions and methods.
CLM What is claimed is:
1. A method for enhancing an immunological response to an OspC antigen in a host comprising: administering to the host at least one OspC antigen with an adjuvant; and administering to the host a lipoprotein selected from the group consisting of OspA, recombinant OspA leader sequence/PspA, recombinant OspA leader sequence/OspC, recombinant OspA leader sequence/UreA and recombinant OspA leader sequence/UreaB, wherein said lipoprotein enhances the immunological response to the OspC

antigen.

2. The method of claim 1 wherein the OspC antigen and the lipoprotein are administered simultaneously.

3. The method of claim 1 wherein the lipoprotein is naturally lipidated.

4. The method of claim 1 wherein the lipoprotein is not naturally lipidated.

5. The method of claim 1 wherein the lipoprotein is an expression product of a hybrid nucleic acid molecule, comprising a first nucleic acid sequence encoding a **signal sequence** of a lipoprotein and a second nucleic acid sequence encoding a mature protein, or immunogenic fragment thereof, which is heterologous to the lipoprotein encoded by the first nucleic acid sequence.

6. The method of claim 5 wherein, in the hybrid nucleic acid molecule, the **signal sequence** is the **signal sequence** of an OspA protein of a Borrelia species, and the sequences are contiguous.

7. The method of claim 6 wherein, in the hybrid nucleic acid molecule, the first nucleic acid sequence and the second nucleic acid sequence are coupled in a translational open reading frame relationship.

8. The method of claim 7 wherein, in the hybrid nucleic acid molecule, the mature protein is an OspC protein of a Borrelia species, or an immunogenic fragment thereof.

9. The method of claim 8 wherein, in the hybrid nucleic acid molecule, the mature protein is an OspC protein from a strain of Borrelia burgdorferi.

10. The method of claim 9 wherein the strain of Borrelia burgdorferi is selected from the B31, ACA1 and Ip90 families of strains.

11. The method of claim 1 wherein the lipoprotein is antigenic.

12. The method of claim 11 wherein the lipoprotein is OspA.

13. The method of claim 1 wherein the antigen and lipoprotein are administered mucosally.

14. The method of claim 13 wherein the antigen and lipoprotein are administered intranasally.

15. The method of claim 13 wherein the antigen and lipoprotein are administered intragastrically.

16. The method of claim 13 wherein the antigen and lipoprotein are administered both intranasally and intragastrically.

17. The method of claim 1 wherein the immunological response is therapeutic.

18. The method of claim 1 wherein the immunological response is prophylactic.

19. The method of claim 5 wherein the second nucleic acid sequence encodes the at least one antigen, whereby the method comprises administering the expression product.

20. The method of claim 8 wherein the second nucleic acid sequence encodes the at least one antigen, whereby the method comprises administering the expression product.

21. The method of claim 20 wherein the lipoprotein is OspA.

22. The method of claim 1 wherein the antigen is OspC and the lipoprotein is OspA.

L6 ANSWER 10 OF 18 USPATFULL on STN
2001:97430 Immunological combination compositions and methods.
Becker, Robert S., Henryville, PA, United States
Huebner, Robert C., Stroudsburg, PA, United States
Gray, Maryann B., Bartonsville, PA, United States
Biscardi, Karen S., South Sterling, PA, United States
Connaught Laboratories, Inc., Swiftwater, PA, United States (U.S.

corporation)
US 6251405 B1 20010626
APPLICATION: US 1995-476656 19950607 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Immunological compositions and methods for making and using them. The compositions contain an antigen and a lipoprotein and optionally an adjuvant. The lipoprotein can itself be antigenic or immurogenic. The antigen can be influenza HA and the lipoprotein a recombinantly expressed product having an OspA leader for lipidation and PspA for the protein portion. The antigen can be OspC and the lipoprotein OspA. The components of the composition are co-administered. A potentiated immunological response is obtained by the compositions and methods.
CLM What is claimed is:
1. An immunological composition comprising at least a first molecule and a second molecule, wherein the first molecule comprises Borrelia OspC antigen and the second molecule comprises an antigenic lipoprotein or lipopolypeptide selected from the group consisting of OspA, recombinant OspA leader sequence/PspA, recombinant OspA leader sequence/OspC, recombinant OspA leader sequence/UreA and recombinant OspA leader sequence/UreB, provided that the antigen and the lipoprotein or lipopolypeptide are in the same physio-chemical form.

2. The composition of claim 1 further comprising an adjuvant.

3. The composition of claim 2 wherein the adjuvant is alum.

4. The composition of claim 2 wherein the antigen is admixed with or adsorbed onto the adjuvant.

5. The composition of claim 2 wherein the lipoprotein or lipopolypeptide is admixed with or adsorbed onto the adjuvant.

6. The composition of claim 2 wherein the antigen is naturally lipidated.

7. The composition of claim 2 wherein the antigen exhibits epitopes of a bacterial protein.

8. The composition of claim 2 wherein the lipoprotein or lipopolypeptide is an expression product of a hybrid nucleic acid molecule, comprising a first nucleic acid sequence encoding a **signal sequence** of a lipoprotein and a second nucleic acid sequence encoding a mature protein, which is heterologous to the lipoprotein encoded by said first nucleic acid sequence.

9. The composition of claim 8 wherein said **signal sequence** is the **signal sequence** of an OspA protein of a Borrelia species, and the sequences are contiguous.

10. The composition of claim 9 wherein said first nucleic acid sequence and said second nucleic acid sequence are coupled in a translational open reading frame relationship.

11. The composition of claim 10 wherein in the hybrid nucleic acid molecule said mature protein is an OspC lipoprotein of a Borrelia species; or said mature protein is PspA.

L6 ANSWER 11 OF 18 USPATFULL on STN
2000:142128 Methods of preparing carboxy-terminally truncated recombinant flavivirus envelope glycoproteins employing drosophila melanogaster expression systems.
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Honolulu, HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6136561 20001024
APPLICATION: US 1997-937195 19970925 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The Flaviviridae comprise a number of medically important pathogens that cause significant morbidity in humans including the dengue (DEN) virus, Japanese encephalitis (JE) virus, tick-borne encephalitis virus (TBE), and yellow fever virus (YF). Flaviviruses are generally transmitted to vertebrates by chronically infected mosquito or tick vectors. The viral particle which is enveloped by host cell membranes, comprises a single positive strand genomic RNA and the structural capsid (CA), membrane

(M), and envelope (E) proteins. The E and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature E is glycosylated and contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. Problems have arisen in the art with respect to producing recombinant forms of the E glycoprotein that retain their native configuration and attendant properties associated therewith (i.e., ability to induce neutralizing antibody responses). To date, recombinantly produced E glycoproteins have suffered from a number of limitations including improper glycosylation, folding, and disulfide bond formation. The claimed invention has addressed these concerns by providing secreted recombinant forms of the E glycoprotein that are highly immunogenic and appear to retain their native configuration. Carboxy-terminally truncated forms of E containing the amino terminal 395 amino acids and a suitable secretion **signal sequence** were generated in Drosophila melanogaster Schneider cell lines. The recombinant proteins produced by this expression system should prove useful, inter alia, as immunogens and diagnostic reagents.

CLM What is claimed is:

1. An expression system for the recombinant production and secretion of a portion of an envelope (E) protein of a Flavivirus selected from the group consisting of dengue virus, **Japanese encephalitis virus** (**JEV**), tick-borne encephalitis virus (TBE) and yellow fever virus (YF), which expression system comprises Drosophila cells modified to contain a DNA molecule which comprises (a) a first nucleotide sequence encoding said portion of said E protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395, and (b) a second nucleotide sequence which encodes a secretory leader sequence or a secretory **signal sequence** operably linked to said first nucleotide sequence and positioned so as to produce a fusion protein when said first and said second nucleotide sequences are expressed in a eucaryotic cell, said encoding sequences operably linked to control sequences capable of effecting expression of said encoding nucleotide sequences in eucaryotic cells.

2. The expression system of claim 1 wherein said secretory leader sequence is human tissue plasminogen activator prepropeptide secretion leader (tPA_L) and optionally includes the premembrane leader of the E protein.

3. A method to produce a portion of an E protein of a Flavivirus selected from the group consisting of dengue virus, **Japanese encephalitis virus** (**JEV**), tick-borne encephalitis virus (TBE) and yellow fever virus (YF), which method comprises (a) culturing the Drosophila cells of claim 1 in culture medium under conditions favorable for expression of the encoding nucleotide sequence so that the cells secrete said portion of the E protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395 into the medium; and (b) recovering the portion of the E protein from the culture medium.

4. A method to produce a portion of an E protein of a Flavivirus selected from the group consisting of dengue virus, **Japanese encephalitis virus** (**JEV**), tick-borne encephalitis virus (TBE) and yellow fever virus (YF), which method comprises (a) culturing the Drosophila cells of claim 2 in culture medium under conditions favorable for expression of the encoding nucleotide sequence so that the cells secrete said portion of the E protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395 into the medium; and (b) recovering the portion of the E protein from the culture medium.

5. The expression system of claim 1 wherein the N-terminal 80% of the E protein from residue 1 to residue 395 is dengue virus E protein.

6. The method of claim 3 wherein the N-terminal 80% of the E protein from residue 1 to residue 395 is dengue virus E protein.

7. The method of claim 4 wherein the N-terminal 80% of the E protein from residue 1 to residue 395 is dengue virus E protein.

8. The expression system of claim 1, wherein the Drosophila cells are Drosophila Schneider cells.

9. The expression system of claim 2, wherein the Drosophila cells are Drosophila Schneider cells.

10. The method of claim 3, wherein the Drosophila cells are Drosophila Schneider cells.

11. The method of claim 4, wherein the Drosophila cells are Drosophila Schneider cells.

12. The expression system of claim 5, wherein the Drosophila cells are Drosophila Schneider cells.

13. The method of claim 6, wherein the Drosophila cells are Drosophila Schneider cells.

14. The method of claim 7, wherein the Drosophila cells are Drosophila Schneider cells.

L6 ANSWER 12 OF 18 USPATFULL on STN
2000:121286 Bioluminescent bioreporter integrated circuit.
Simpson, Michael L., Knoxville, TN, United States
Sayler, Gary S., Blaine, TN, United States
Paulus, Michael J., Knoxville, TN, United States
UT Battelle, LLC, Oak Ridge, TX, United States (U.S. corporation)The University of Tennessee Research Corp., Knoxville, TX, United States (U.S. corporation)
US 6117643 20000912
APPLICATION: US 1997-978439 19971125 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are monolithic bioelectronic devices comprising a bioreporter and an OASIC. These bioluminescent bioreporter integrated circuit are useful in detecting substances such as pollutants, explosives, and heavy-metals residing in inhospitable areas such as groundwater, industrial process vessels, and battlefields. Also disclosed are methods and apparatus for environmental pollutant detection, oil exploration, drug discovery, industrial process control, and hazardous chemical monitoring.
CLM What is claimed is:
1. An apparatus for detecting the concentration of a selected substance, comprising: (a) a substrate; (b) a bioreporter capable of metabolizing or interacting with a selected substance to emit light; (c) a selectively permeable container affixed to said substrate capable of holding said bioreporter; and (d) an integrated circuit on said substrate, including a phototransducer operative to generate a **signal** in response to said light.

2. The apparatus of claim 1 wherein the **signal** of step (d) indicates the presence of said substance.

3. The apparatus of claim 2, wherein said bioresistant/biocompatible material comprises silicon nitride.

4. The apparatus of claim 1, further comprising a layer of bioresistant and biocompatible material between said substrate and said container.

5. The apparatus of claim 1, wherein said integrated circuit is a Complementary Metal Oxide Semiconductor (CMOS) integrated circuit.

6. The apparatus of claim 1, wherein said phototransducer comprises a photodiode.

7. The apparatus of claim 1, wherein said integrated circuit further comprises a current to frequency converter and a digital counter.

8. The apparatus of claim 1, wherein said integrated circuit further comprises a photodiode and a current to frequency converter.

9. The apparatus of claim 1, wherein said integrated circuit further comprises a transmitter.

10. The apparatus of claim 9, wherein said transmitter is wireless.

11. The apparatus of claim 9, further comprising a receiver capable of receiving transmissions from said transmitter.

12. The apparatus of claim 11, wherein transmissions comprise digital data.

13. The apparatus of claim 1, further comprising a fluid and nutrient reservoir and a microfluidic pump on said substrate.

14. The apparatus of claim 1, wherein said bioreporter is selected from

a group consisting of a genetically-engineered yeast, bacterium, fungal, animal and plant cell.

15. The apparatus of claim 1, wherein said selectively permeable container comprises a polymer matrix.

16. The apparatus of claim 15 wherein said polymer matrix is capable of allowing gas or fluid to reach said bioreporter.

17. The apparatus of claim 15, wherein said polymer matrix is optically clear.

18. The apparatus of claim 1, wherein said integrated circuit further comprises a global positioning system.

19. A monolithic bioelectronic device for detecting a substance, which comprises: (a) a bioreporter atop a substrate on an integrated circuit, said bioreporter being capable of metabolizing the substance and emitting light consequent to such metabolism when in contact with said substance; and, (b) a sensor closely positioned to said integrated circuit to generate an electrical **signal** in response to receiving the emitted light.

20. The monolithic bioelectronic device of claim 19, wherein the bioreporter comprises a nucleic-acid segment encoding a bioluminescent marker polypeptide expressed in the presence of the substance.

21. The monolithic bioelectronic device of claim 20, wherein the sequence of the nucleic-acid segment encodes a luminescent reporter molecule.

22. The monolithic bioelectronic device of claim 19, further comprising a transparent, bioresistant and biocompatible separator positioned between the bioreporter and the sensor.

23. The monolithic bioelectronic device of claim 19, further comprising a polymer matrix encasing the bioreporter and enabling contact between the substance and the bioreporter.

24. The monolithic bioelectronic device of claim 23, wherein the polymer matrix is permeable to the substance.

25. The monolithic bioelectronic device of claim 19, wherein the bioreporter is identified as a microorganism.

26. The monolithic bioelectronic device of claim 25, which further comprises a source of water and nutrients suitable for sustaining the bioreporter.

27. Apparatus for detecting a substance which comprises: (a) an integrated circuit including a phototransducer that produces a **signal** in response to light; (b) a bioreporter capable of metabolizing the substance and emitting light consequent to such metabolism, wherein said reporter is in contact with said substance; and (c) a transparent, biocompatible, and bioresistant separator positioned between the phototransducer and the bioreporter.

28. Apparatus as defined in claim 27, wherein the bioreporter comprises a nucleic-acid sequence encoding a lux gene, wherein said gene expresses a light-emitting polypeptide concurrently with the metabolism of said substance.

29. Apparatus as defined in claim 27 which further comprises a plastic matrix encasing the bioreporter and enabling contact between the substance and the bioreporter.

30. Apparatus as defined in claim 29 wherein the plastic matrix is permeable to the substance.

31. Apparatus as defined in claim 30 wherein the substance comprises a fluid.

32. Apparatus as defined in claim 27 in which the bioreporter comprises bacteria.

L6 ANSWER 13 OF 18 USPATFULL on STN
2000:47031 Non-A, non-B, non-C, non-D, non-E hepatitis reagents and methods for their use.

Simons, John N., Grayslake, IL, United States
Pilot-Matias, Tami J., Green Oaks, IL, United States
Dawson, George J., Libertyville, IL, United States
Schlauder, George G., Skokie, IL, United States
Desai, Suresh M., Libertyville, IL, United States
Leary, Thomas P., Kenosha, WI, United States
Muerhoff, Anthony Scott, Kenosha, WI, United States
Erker, James Carl, Hainesville, IL, United States
Buijk, Sheri L., Round Lake, IL, United States
Mushahwar, Isa K., Grayslake, IL, United States
Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)
US 6051374 20000418
APPLICATION: US 1995-488445 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Hepatitis GB Virus (HGBV) nucleic acid and amino acid sequences useful for a variety of diagnostic and therapeutic applications, kits for using the HGBV nucleic acid or amino acid sequences, HGBV immunogenic particles, and antibodies which specifically bind to HGBV. Also provided are methods for producing antibodies, polyclonal or monoclonal, from the HGBV nucleic acid or amino acid sequences.

CLM What is claimed is:

1. A method for detecting target hepatitis GB virus (HGBV) nucleic acid in a test sample suspected of containing HGBV comprising: (a) reacting the test sample with an HGBV polynucleotide probe comprising a sequence of at least 15 contiguous nucleotides, wherein said sequence selectively hybridizes to a genome of hepatitis GB virus (HGBV) or its full complement, wherein said HGBV comprises a positive stranded RNA viral genome comprising an open reading frame encoding a polyprotein, wherein said polyprotein (i) has an amino acid sequence having at least 35% identity to a polyprotein sequence selected from the group consisting of SEQ ID NO:387, SEQ ID NO:394, and SEQ ID NO:401 wherein said % identity is determined by using the computer program GAP of the Wisconsin Package, Version 8 with the gap penalty set to 3 and the gap extension penalty set to 0.1; and (ii) is not immunoreactive with an antibody that specifically reacts with a protein encoded by a virus selected from the group consisting of hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis delta virus and hepatitis E virus, under conditions and for a time which allows the formation of a complex between the probe and the target HGBV nucleic acid in the test sample; and (b) detecting the complex that contains the target HGBV nucleic acid wherein the presence of the complex is indicative of the presence of the target HGBV.

2. The method of claim 1, wherein the target HGBV is amplified prior to performing step (a).

3. The method of claim 2, wherein said amplifying is performed by the polymerase chain reaction or the ligase chain reaction.

4. The method of claim 1, wherein said polynucleotide probe further comprises a measurable **signal** generating compound.

5. The method of claim 4, wherein said **signal** generating compound is selected from the group consisting of a chemiluminescent compound, fluorescein, an enzyme, and a radioactive element.

6. The method of claim 2, wherein said target is immobilized on a solid phase.

7. The method of claim 4, wherein said target is immobilized on a solid phase.

8. The method of claim 2, wherein said amplifying further comprises utilizing a primer conjugated to a first hapten.

9. The method of claim 8, further comprising contacting said complex with said primer-first hapten under conditions and for a time which allows the formation of a complex between the probe/target and the primer-first hapten.

10. The method of claim 9, further comprising contacting said probe/target/first hapten complex with an anti-first hapten antibody conjugated to a detectable **signal** generating compound prior to performing step (b).

11. The method of claim 10, wherein said **signal** generating compound is selected from the group consisting of a chemiluminescent compound, fluorescein, an enzyme, and a radioactive element.

12. The method of claim 1, wherein said probe is immobilized on a solid phase.

13. The method of claim 12, wherein said probe is immobilized to said solid phase prior to performing step (a).

14. The method of claim 8, wherein said first hapten is selected from the group consisting of adamatane, carbazole, fluorescein, and biotin.

15. The method of claim 14, wherein said polynucleotide probe is conjugated to a second hapten, with the proviso that the second hapten is different than the first hapten.

16. The method of claim 15, further comprising reacting said probe with a solid phase to which an anti-second hapten antibody conjugated to said probe is immobilized, prior to performing step (b).

17. The method of claim 16, further comprising contacting an anti-first hapten antibody conjugated to a measurable **signal** generating compound with said probe/target complex prior to performing step (b).

18. The method of claim 17, wherein said **signal** generating compound is selected from the group consisting of a chemiluminescent compound, fluorescein an enzyme, and a radioactive element.

19. The method of claim 2, wherein said polynucleotide probe is conjugated to a hapten.

20. The method of claim 19, wherein said hapten is selected from the group consisting of adamatane, carbazole, fluorescein, and biotin.

21. The method of claim 19, further comprising immobilizing said target to a solid phase prior to performing step (b).

22. A test kit useful for determining the presence of target hepatitis GB virus (HGBV) nucleic acid in a test sample, comprising a container containing a polynucleotide probe wherein said polynucleotide probe comprises a sequence of at least 15 contiguous nucleotides, and wherein said polynucleotide probe selectively hybridizes to a genome of hepatitis GB (HGBV) or its full complement, wherein said HGBV comprises a positive stranded RNA viral genome comprising an open reading frame encoding a polyprotein, wherein said polyprotein (a) has an amino acid sequence having at least 35% identity to a polyprotein sequence selected from the group consisting of SEQ ID NO:387, SEQ ID NO:394, and SEQ ID NO:401 wherein said % identity is determined by using the computer program GAP of the Wisconsin Package, Version 8 with the gap penalty set to 3 and the gap extension penalty set to 0.1; and (b) is not immunoreactive with an antibody that specifically reacts with a protein encoded by a virus selected from the group consisting of hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis delta virus, and hepatitis E virus.

23. The test kit of claim 22, wherein said polynucleotide probe further comprises a measurable **signal** generating compound.

24. The test kit of claim 22, wherein said **signal** generating compound is selected from the group consisting of a chemiluminescent compound, fluorescein an enzyme, and a radioactive element.

25. The test kit of claim 22, wherein said polynucleotide probe further comprises a hapten.

26. The test kit of claim 25, wherein said hapten is selected from the group consisting of adamatane, carbazole, fluorescein, and biotin.

27. The test kit of claim 22, further comprising a container containing at least one primer.

28. The test kit of claim 27, wherein said primer further comprises a hapten.

29. The test kit of claim 28, wherein said hapten is selected from the group consisting of adamatane, carbazole, fluorescein, and biotin.

30. The test kit of claim 28 wherein said test kit further comprises a solid phase to which a second hapten is immobilized.

31. The test kit of claim 30, wherein said second hapten is selected from the group consisting of adamatane, carbazole, fluorescein, and

biotin, with the proviso that the second hapten is different than the first hapten.

32. The method of claim 1, wherein said encoded polyprotein has an amino acid sequence having at least 40% identity to a polyprotein selected from the group consisting of SEQ ID NO:387, SEQ ID NO:394, and SEQ ID NO:401.

33. The test kit of claim 22, wherein said encoded polyprotein has an amino acid sequence having at least 40% identity to a polyprotein selected from the group consisting of SEQ ID NO:387, SEQ ID NO:394, and SEQ ID NO:401.

34. The method of claim 1, wherein said encoded polyprotein has an amino acid sequence having at least 60% identity to a polyprotein selected from the group consisting of SEQ ID NO:387, SEQ ID NO:394, and SEQ ID NO:401.

35. The test kit of claim 22, wherein said encoded polyprotein has an amino acid sequence having at least 60% identity to a polyprotein selected from the group consisting of SEQ ID NO:387, SEQ ID NO: 394, and SEQ ID NO:401.

L6 ANSWER 14 OF 18 USPATFULL on STN

1999:102667 Method and system for enhanced production of commercially important exoproteins in gram-positive bacteria.

Kontinen, Vesa, Helsinki, Finland
Sarvas, Matti, Helsinki, Finland
The Finnish National Public Health Institute, Helsinki, Finland (non-U.S. corporation)

US 5945278 19990831
APPLICATION: US 1998-108920 19980701 (9)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides a method and expression system for enhancing secretion of hyperproduced homologous and heterologous exoproteins in gram-positive bacteria such as Bacillus sp. The method and system comprise overproduction of PrsA protein in a gram-positive bacterial host also overproducing at least one exoprotein of interest. Use of the method and system of the invention results in greatly enhanced secretion of the synthesized exoproteins into the growth medium. Once in the growth medium these secreted exoproteins can be recovered and purified in a straightforward manner.

CLM What is claimed is:

1. An expression system for enhancing secretion of exoproteins in gram-positive bacteria engineered to express greater than wild-type amounts of PrsA protein, or functional homologues thereof, wherein said gram-positive bacteria express greater than wild-type amounts of at least one exoprotein of interest.

2. An expression system according to claim 1 wherein said PrsA protein is endogenous to said gram-positive bacteria.

3. An expression system according to claim 1 wherein said PrsA protein is heterologous to said gram-positive bacteria.

4. An expression system according to claim 1 wherein said PrsA protein is PrsA protein from a species of Bacillus.

5. An expression system according to claim 1 wherein said functional homologue of said PrsA protein is immunologically reactive with antibody raised against PrsA protein from Bacillus subtilis, Bacillus amyloliquefaciens, or Bacillus licheniformis, and when overexpressed, enhances secretion of said exoprotein of interest from said gram-positive bacteria.

6. An expression system according to claim 1 wherein said PrsA protein, or functional homologue thereof, is present in said gram-positive cell in amounts that are from 2 to about 10 times greater than wild-type amounts.

7. An expression system according to claim 1 wherein said exoprotein of interest is a protease, a lipase, a cutinase, an amylase, a galactosidase, a pullulanase, a cellulase, a glucose isomerase, a protein disuphide isomerase, a CGT'ase (cyclodextrin gluconotransferase), a phytase, a glucose oxidase, a glucosyl transferase, laccase, bilirubin oxidase, a xylanase, an antigenic microbial or protozoan protein, a bacterial protein toxin, a microbial

surface protein, a viral protein, or a pharmaceutical.

8. An expression system according to claim 1 wherein said gram-positive bacteria is a species of Bacillus.

9. A method for identifying a gene which encodes a functional homologue of PrsA from Bacillus subtilis, said method comprising identifying protein that reacts with anti-PrsA antibodies of high titer, demonstrating that when said protein is present in greater than wild-type amounts in a gram-positive bacteria, said protein enhances the secretory capability of said gram-positive bacteria with respect to secretion of an exoprotein of interest wherein said gram-positive bacteria contains a gene which encodes a functional homologue of PrsA from Bacillus subtilis.

10. An expression system according to claim 4 wherein said PrsA protein is PrsA protein from Bacillus subtilis, Bacillus amyloliquefaciens or Bacillus licheniformis.

11. An expression system according to claim 7 wherein said exoprotein of interest is a non native exoprotein that has been created by the addition of a **signal sequence** to the structural gene encoding said protein.

12. An expression system according to claim 8 wherein said Bacillus is Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulàns, Bacillus lautus or Bacillus thuringiensis.

13. A gram-positive bacteria expressing greater than wild-type amounts of at least one exoprotein of interest further comprising pKTH277.

14. A gram-positive bacteria expressing greater than wild-type amounts of at least one exoprotein of interest and further comprising at least one of the following: at least two copies of the prsA gene from Bacillus subtilis, or a functional homologue thereof; the prsA gene from Bacillus subtilis, or a functional homologue thereof, operatively linked to strong regulatory sequences which result in overexpression of said prsA gene, or functional homologue thereof.

15. A gram-positive bacteria according to claim 14 wherein said gram-positive bacteria is a bacteria from the genus Bacillus.

16. A gram-positive bacteria according to claim 15 wherein said Bacillus is Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus or Bacillus thuringiensis.

17. A method for enhancing secretion of an exoprotein of interest in a gram-positive bacteria comprising expressing greater than wild type amounts of PrsA protein from Bacillus, or a functional homologue thereof in said gram-positive bacteria, wherein said gram-positive bacteria also expresses greater than wild type amounts of said exoprotein.

18. A method according to claim 17 wherein said gram-positive bacteria is a bacteria from the genus Bacillus.

19. A method according to claim 18 wherein said Bacillus bacteria is Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus or Bacillus thuringiensis.

20. An expression system for enhancing secretion of exoproteins in gram-positive bacteria comprising a gram-positive bacteria expressing greater than wild-type amounts of PrsA protein and expressing greater than wild-type amounts of at least one exoprotein of interest.

21. A method for identifying a gene which encodes a functional homologue of PrsA from Bacillus subtilis, said method comprising identifying, by means of Southern blotting, DNA which hybridizes with DNA probe(s) from the prsA gene from Bacillus subtilis, and demonstrating that the gene encodes a protein which when overexpressed, enhances the secretory capability of a gram-positive bacteria with respect to secretion of an exoprotein of interest.

L6 ANSWER 15 OF 18 USPATFULL on STN
1998:82562 Method and system for enhanced production of commercially important exoproteins in gram-positive bacteria.
Kontinen, Vesa, Helsinki, Finland
Sarvas, Matti, Helsinki, Finland
The Finnish National Public Health Institute (KTL), Helsinki, Finland (non-U.S. corporation)
US 5780261 19980714
WO 9419471 19940901
APPLICATION: US 1996-507391 19960708 (8)
WO 1994-FI72 19940225 19960708 PCT 371 date 19960708 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides a method and expression system for enhancing secretion of hyperproduced homologous and heterologous exoproteins in gram-positive bacteria such as Bacillus sp. The method and system comprise overproduction of PrsA protein in gram-positive bacterial host capable of also overproducing at least one exoprotein of interest. Use of the method and system of the invention results in greatly enhanced secretion of the synthesized exoproteins into the growth medium. Once in the growth medium these secreted exoproteins can be recovered and purified in a straightforward manner.

CLM What is claimed is:

1. An expression system for enhancing secretion of exoproteins in gram-positive bacteria engineered to express greater than wild-type amounts of PrsA protein from Bacillus, wherein said gram-positive bacteria express greater than wild-type amounts of at least one exoprotein of interest.

2. An expression system according to claim 1 wherein said PrsA protein is heterologous to said gram-positive bacteria.

3. An expression system according to claim 1, wherein said PrsA protein is PrsA protein from Bacillus subtilis, Bacillus amyloliquefaciens or Bacillus licheniformis.

4. An expression system according to claim 1 wherein said PrsA protein is present in said gram-positive bacteria in amounts that are from 2 to about 10 times greater than wild-type amounts.

5. An expression system according to claim 1 wherein said exoprotein of interest is a protease, a lipase, a cutinase, an amylase, a galactosidase, a pullulanase, a cellulase, a glucose isomerass, a protein disuphide isomerase, a CGT'ass (cyclodextrin gluconotransferase), a phytase, a glucose oxidase, a glucosyl transferase, laccase, bilirubin oxidase, a xylanase, an antigenic microbial or protozoan protein, a bacterial protein toxin, a microbial surface protein, a viral protein, a pharmaceutical.

6. An expression system according to claim 5 wherein said exoprotein of interest is a non native exoprotein that has been created by the addition of a **signal sequence** to the structural gene encoding said protein.

7. An expression system according to claim 1 wherein said gram-positive bacteria is a species of Bacillus.

8. An expression system according to claim 7 wherein said Bacillus is Bacillus subtills, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophllus, Bacillus alkalophilus, Bacillus amyloliquefeciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus and Bacillus thuringlensis.

9. A gram-positive bacteria expressing greater than wild-type amounts of at least one exoprotein of interest further comprising pKTH277.

10. A gram-positive bacteria expressing greater than wild-type amounts of at least one exoprotein of Interest and further comprising at least one of the following: (1) at least two copies of the prsA gene from Bacillus subtilis, (2) the prsA gone from Bacillus subtilis, operatively linked to strong regulatory sequences which result in overexpression of said prsA gene.

11. A gram-positive bacteria according to any of claims 9 or 10 wherein said gram-positive bacteria is a bacteria from the genus Bacillus.

12. A gram-positive bacteria according to claim 14 wherein said Bacillus is Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus bravis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus

amylollquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus and Bacillus thuringlensis.

13. A method for enhancing secretion of an exoprotein of interest in a gram-positive bacteria comprising expressing greater than wild type amounts of PrsA protein from Bacillus, in said gram-positive bacteria, wherein said gram-positive bacteria also expresses greater than wild type amounts of said exoprotein.

14. A method according to claim 13 wherein said gram-positive bacteria is a bacteria from the genus Bacillus.

15. A method according to claim 14 wherein said Bacillus bacteria is Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefacions, Bacillus coagulans, Bacillus circulans, Bacillus lautus or Bacillus thuringlensis.

16. An expression system for enhancing secretion of exoproteins in gram-positive bacteria comprising a gram-positive positive bacteria expressing greater than wild-type amounts of PrsA protein and expressing greater than wild-type amounts of at least one exoprotein of interest.

L6 ANSWER 16 OF 18 USPATFULL on STN
95:3771 Expression vectors and methods for intracellular protein production in bascillus.
Palva, Ilkka, Helsinki, Finland
The Finnish National Public Health Institute, Finland (non-U.S. corporation)
US 5380653 19950110
APPLICATION: US 1992-947888 19920918 (7)
PRIORITY: FI 1980-4081 19801231
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to recombinant DNA molecules and to methods for producing proteins by means of said molecules. Particularly, the present invention relates to recombinant DNA molecules which are capable of being synthesized in Bacillus strain bacteria comprising the regulation and deleted non-functional **signal sequence** of the a-amylase gene of B. amyloliquefaciens, or a substantial part thereof, to which sequence a structural gene of any desired homologous or heterologous protein or peptide may be joined. These recombinant DNA molecules can be used, for example, to achieve intracellular expression of any desired protein or peptide in Bacillus strain bacteria.
CLM What is claimed is:
1. A recombinant DNA molecule comprising (1) the regulation sequence of the α-amylase gene of Bacillus amyloliquefaciens, (2) DNA encoding a truncated, non-functional **signal sequence** of the α-amylase gene of Bacillus amyloliquefaciens wherein said DNA comprises DNA encoding at least the N-terminal initiation methionine (Met) and the next six adjacent N-terminal amino acids of the wild type of said **signal sequence**, but not DNA encoding the seven C-terminal amino acids of the wild type of said **signal sequence**, and (3) DNA encoding amino acids of a desired protein or polypeptide wherein said DNA encoding said desired protein or polypeptide Is downstream from and in phase with said regulation sequence and said DNA encoding said non-functional **signal sequence**.

2. A recombinant DNA molecule of claim 1 wherein said truncated, non-functional **signal sequence** is from a plasmid selected from the group consisting of pKTH 39, pKTH 1781 and pKTH 1784 as shown in FIG. 5.

3. A recombinant DNA molecule of claim 1, wherein said truncated, non-functional **signal sequence** comprises amino acids encoded by ATG ATT CAA AAA CGA AAG CGG.

4. A recombinant DNA molecule of claim 1, wherein said truncated, non-functional **signal sequence** comprises amino acids encoded by ATG ATT CAA AAA CGA AAG CGG AAT TCC.

5. A recombinant DNA molecule of claim 1, wherein said truncated, non-functional **signal sequence** comprises amino acids encoded by ATG ATT CAA AAA CGA AAG CGG AAT TCG GAA GCT TXX.

6. A recombinant DNA molecule of claim 1, wherein said truncated, non-functional **signal sequence** comprises amino acids encoded by ATG ATT CAA AAA CGA AAG CGG AAT TTA AGC TTX.

7. A recombinant DNA molecule of claim 1 wherein said DNA encoding amino acids of a desired protein or polypeptide is selected from the group consisting of DNA encoding chloramphenicol acetyl transferase and DNA encoding pertussis toxin or subunits thereof.

8. A vector comprising a recombinant DNA molecule of claim 1.

9. The vector of claim 8, wherein said DNA encoding amino acids of a desired protein or polypeptide is selected from the group consisting of DNA encoding chloramphenicol acetyl transferase and DNA encoding pertussis toxin or subunits thereof.

10. A Bacillus host comprising the vector of claim 8.

11. A Bacillus host comprising the vector of claim 9.

12. A method for obtaining intracellular expression of a desired homologous or heterologous protein, or polypeptide, in a Bacillus host, comprising (a) transforming a desired Bacillus host with a recombinant DNA molecule comprising (1) the regulation sequence of the α-amylase gene of Bacillus amyloliquefaciens, (2) DNA encoding a truncated, non-functional **signal sequence** of the α-amylase gene of Bacillus amyloliquefaciens wherein said DNA comprises DNA encoding at least the N-terminal initiation methionine (Met) and the next six adjacent N-terminal amino acids of the wild type of said **signal sequence**, but not DNA encoding the seven C-terminal amino acids of the wild type of said **signal sequence** and (3) DNA encoding amino acids of a desired protein or polypeptide wherein said DNA encoding said desired protein or polypeptide is downstream from and in phase with said regulation sequence and said DNA encoding said non-functional **signal sequence**, and (b) culturing the Bacillus host of step (a) under conditions allowing Intracellular expression of said desired homologous or heterologous protein or polypeptide.

L6 ANSWER 17 OF 18 USPATFULL on STN
91:32387 Recombinant DNA-molecules and method for protein production.
Palva, Ilkka, Helsinki, Finland
Genesit Oy, Helsinki, Finland (non-U.S. corporation)Oy Alko AB, Helsinki, Finland (non-U.S. corporation)
US 5010015 19910423
APPLICATION: US 1987-129357 19871130 (7)
PRIORITY: FR 1980-4081 19801231
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to recombinant DNA molecules and to methods for producing proteins by means of said molecules. Particularly, the present invention relates to recombinant DNA molecules which are synthetized in Bacillus strain bacteria and are characterized by DNA which codes for exoenzymes excreted by a bacterium of the Bacillus strain and which are present in tens of copies in Bacillus strain bacteria, as well as to recombinant DNA molecules which are modified from the above recombinant DNA molecules and are characterized by DNA containing the regulation and excretion signals of the α-amylase gene of B. amyloliquefaciens, to which signals a gene of any protein can be joined. These recombinant DNA molecules can be used, for example, to improve the production of α-amylase in Bacillus strain bacteria, and their modifications to produce any protein in Bacillus strain bacteria.

CLM What is claimed is:
1. A recombinant DNA molecule comprising a plasmid which is capable of multiplying in Bacillus strain bacteria, the regulation and secretion **signal** sequences of the α-amylase gene of B. amyloliquefaciens, and the DNA sequence encoding the amino acids of a desired protein or polypeptide, said DNA sequence being downstream of said secretion **signal sequence**, with the proviso that said desired protein is not α-amylase of B. amyloliquefaciens.

2. A recombinant DNA molecule as claimed in claim 1 in which said DNA sequence encoding the amino acids of the desired protein codes for an interferon selected from the group consisting of α-interferon and β-interferon.

3. A recombinant DNA molecule as claimed in claim 1 in which said DNA sequence codes for β-lactamase of E. coli.

4. A recombinant DNA molecule as claimed in claim 1 in which the plasmid contained in said DNA molecule is pUB110.

5. A recombinant DNA molecule according to claim 1 wherein said regulation and secretion **signal** sequences of the α-amylase gene has the following nucleotide sequence: ##STR2##

6. A recombinant DNA molecule as claimed in claim 1 in which the DNA sequence coding for said desired protein or polypeptide is joined to a DNA sequence selected from the group consisting of

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5' CTG TTA TTT GTC AGT TTG CCG ATT ACA AAA ACA TCA GCC,
5' CTG TTA TTT GTC AGT TTG CCG ATT ACA AAA ACA TCA GCC G,
5' CTG TTA TTT GTC AGT TTG CCG ATT ACA AAA ACA TCA GCC GT,
5' CTG TTA TTT GTC AGT TTG CCG ATT ACA AAA ACA TCA GCC GTA,
5' CTG TTA TTT GTC AGT TTG CCG ATT ACA AAA ACA TCA GCC GTA A, and
5' CTG TTA TTT GTC AGT TTG CCG ATT ACA AAA ACA TCA GCC GTA

AA.

L6 ANSWER 18 OF 18 USPATFULL on STN
91:32372 Method for the preparation of a selected protein or a part thereof in Bacillus strain bacteria.
Palva, Ilkka, Helsinki, Finland
Genesit Oy, Helsinki, Finland (non-U.S. corporation)Oy Alko AB, Helsinki, Finland (non-U.S. corporation)
US 5010000 19910423
APPLICATION: US 1987-129356 19871130 (7)
PRIORITY: FI 1980-4081 19801231
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A selected protein or protein part is prepared in Bacillus strain bacteria by joining DNA encoding the selected protein or protein part essential to its biological activity to a bacterium gene. The Bacillus amyloliquefaciens gene for α-amylase is cleaved at a location after the excretion signal following the regulation sequence, or at a location after a part essential with respect to its excretion. The cleaved gene is joined to a plasmid present in Bacillus strain bacteria in several copies, and the DNA sequence encoding the selected protein or protein part essential to its biological activity is joined to the cleavage site by recombinant DNA techniques. Bacillus strain host bacteria are transformed with the recombinant DNA molecules so obtained, and the transformed host bacteria cultivated to produce the selected protein or protein part.

CLM What is claimed is:
1. A method for the preparation of a selected protein or polypeptide in Bacillus strain bacteria by joining DNA coding for the selected protein or polypeptide to a bacterium gene, comprising the successive steps of: (1) cleaving the Bacillus amyloliquefaciens gene for α-amylase at a location to the 3' side of the excretion **signal** downstream from the regulation **signal** of the α-amylase gene or at a location downstream from a portion of the DNA sequence essential with respect to excretion of the α-amylase protein; (2) joining the cleaved gene to a plasmid present in Bacillus strain bacteria; (3) joining the DNA sequence coding for the selected protein or polypeptide to the cleavage site; (4) transforming the Bacillus strain host bacteria with the recombinant DNA molecules so obtained; and (5) cultivating the transformed host bacteria for producing the selected protein or polypeptide.

2. A method as claimed in claim 1 in which the DNA sequence coding for the amino acids in the selected protein or polypeptide is joined to a nucleotide sequence selected from the group consisting of: ##STR2##

3. A method as claimed in claim 1 in which the selected protein or polypeptide is any of the α- or β-interferons.

4. A method as claimed in claim 1 in which the selected protein or polypeptide is E. coli β-lactamase.

5. A method as claimed in claim 1 in which the host bacterium is B. subtilis.

6. A method for the preparation of a selected protein or polypeptide in a Bacillus subtilis host, comprising the steps of joining DNA coding for the selected protein or polypeptide to a recombinant plasmid vector obtained by cleaving a gene encoding a Bacillus strain protein or

polypeptide at a location to the 3' side of the excretion **signal** downstream from the regulation **signal**, or at a location downstream from a portion of the DNA sequence essential with respect to the excretion of said Bacillus strain protein or polypeptide, joining the cleaved gene to a plasmid present in Bacillus strain bacteria, transforming the Bacillus subtilis host with the recombinant DNA molecules so obtained, and cultivating the transformed host to produce excretable protein or polypeptide, wherein the DNA sequence coding for the selected protein or polypeptide is joined to a recombinant plasmid vector comprising a plasmid present in Bacillus strain bacteria, and the regulation **signal** of the gene coding for Bacillus amyloliquefaciens α-amylase and the excretion **signal** or a part thereof essential with respect to the excretion of said α-amylase.

7. A method as claimed in claim 6 in which the DNA sequence coding for the selected protein or polypeptide is a gene coding for any of the α- or β-interferons.

8. A method as claimed in claim 6 in which the DNA sequence coding for the selected protein or polypeptide is a gene coding for E. coli β-lactamase.

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=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      73.52      73.73

FILE 'MEDLINE' ENTERED AT 19:49:17 ON 25 NOV 2005

 FILE LAST UPDATED: 25 NOV 2005 (20051125/UP).  FILE COVERS 1950 TO DATE.

 On December 19, 2004, the 2005 MeSH terms were loaded.

 The MEDLINE reload for 2005 is now available.  For details enter HELP
 RLOAD at an arrow promt (=>).  See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html

 OLDMEDLINE now back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2005 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> e chang g j j/au
E1            9     CHANG G H/AU
E2           50     CHANG G J/AU
E3            0 --> CHANG G J J/AU
E4            2     CHANG G K/AU
E5           22     CHANG G L/AU
E6            1     CHANG G M/AU
E7            7     CHANG G N/AU
E8           11     CHANG G Q/AU
E9            2     CHANG G R/AU
E10           3     CHANG G S/AU
E11          28     CHANG G T/AU
E12           3     CHANG G T G/AU

=> s e2
L7           50 "CHANG G J"/AU

=> d l7,ti

L7   ANSWER 1 OF 50     MEDLINE on STN
TI   Detection of yellow fever virus by polymerase chain reaction.


=> s l7 and signal
        273291 SIGNAL
L8           3 L7 AND SIGNAL

=> d l8,ti,1-3
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L8 ANSWER 1 OF 3 MEDLINE on STN
TI Detection of yellow fever virus by polymerase chain reaction.

L8 ANSWER 2 OF 3 MEDLINE on STN
TI Flavivirus DNA vaccines: current status and potential.

L8 ANSWER 3 OF 3 MEDLINE on STN
TI An integrated target sequence and **signal** amplification assay, reverse transcriptase-PCR-enzyme-linked immunosorbent assay, to detect and characterize flaviviruses.

=> d l8,cbib,ab,1-3

L8 ANSWER 1 OF 3 MEDLINE on STN
2004593406. PubMed ID: 15566752. Detection of yellow fever virus by polymerase chain reaction. Brown T M; **Chang G J**; Cropp C B; Robbins K E; Tsai T F. (Division of Vector-Borne Infectious Diseases, National Centers for Infectious Diseases, Centers for Disease Control, P.O. Box 2087, Fort Collins, CO 80522, USA.) Clinical and diagnostic virology, (1994 Feb) 2 (1) 41-51. Journal code: 9309653. ISSN: 0928-0197. Pub. country: Netherlands. Language: English.
AB BACKGROUND: Yellow fever virus continues to cause major epidemics. A sensitive rapid diagnostic test is required to identify cases and contacts in order to implement emergency immunization campaigns. OBJECTIVES: To identify YFV envelope protein gene fragments, construct a polymerase chain reaction (PCR) assay and test its utility in identifying viruses isolated from laboratory and clinical specimens. STUDY DESIGN: YFV RNA was transcribed with reverse transcriptase and the cDNA amplified by PCR using primers encoding a portion of the viral envelope protein gene. The identity of the 482 bp amplified product was confirmed by restriction enzyme analysis and by dot blot hybridization with a labelled oligonucleotide probe. The assay was tested for sensitivity and specificity on isolates from South America and Africa. Detection limits were determined using different probe labels. PCR inhibitory effects were analyzed with laboratory and clinical specimens. RESULTS: The assay was specific for YFV and did not detect any of 15 other flaviviruses. The amplified region was conserved among all 32 South American and African isolates tested. Four strains from Africa did not hybridize with the probe, indicating sequence divergence in the envelope protein gene. Samples containing 30 pfu of virus were detected by visual inspection of the ethidium bromide stained 482 bp DNA amplimer and 10 pfu were detected with a digoxigenin labelled probe. Inhibitory effects of human serum on the PCR were overcome by diluting samples 4-fold in buffer. Viral neutralizing antibody in experimental samples did not affect the sensitivity of detection. Yellow fever virus in serum from experimentally infected Cynomolgus monkeys (10(3.7)-10(7.0) pfu/0.1 ml) was detected with **signal** intensities corresponding to the amount of virus in the sample. When YFV was added to normal human serum and held at 27 degrees C and 80% humidity, the RNA could be detected for up to 3 weeks in samples that had no infectious virus. CONCLUSIONS: A PCR assay was constructed which detected YFV RNA in isolates from patients infected in South America and Africa. This assay is specific for YFV but some African strains were not detected. More clinical samples should be tested.

L8 ANSWER 2 OF 3 MEDLINE on STN
2002071990. PubMed ID: 11797784. Flavivirus DNA vaccines: current status and potential. **Chang G J**; Davis B S; Hunt A R; Holmes D A; Kuno G. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522, USA.. gxc7@cdc.gov) . Annals of the New York Academy of Sciences, (2001 Dec) 951 272-85. Ref: 54. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.
AB The use of DNA-based vaccines is a novel and promising immunization approach for the development of flavivirus vaccines. This approach has been attempted in vaccine development for various virus species, including St. Louis encephalitis, Russian spring-summer encephalitis, Central European encephalitis, dengue serotypes 1 and 2, Murray Valley encephalitis, Japanese encephalitis, and West Nile viruses. However, very little is known about the factors affecting its efficacy. Recently, we demonstrated that a single intramuscular immunization of DNA vaccine of Japanese encephalitis and West Nile viruses protected mice and horses from virus challenge. Administration of these recombinant plasmid vectors resulted in endogenous expression and secretion of extracellular virus-like particles that correlated well with the induction of protective immunity. These results provided evidence that the virus-like particles composed of premembrane/membrane and envelope proteins are essential for eliciting immune responses similar to those induced by live, attenuated virus vaccines. The biosynthesis and protein processing of

premembrane/membrane and envelope proteins that preserve the native conformation and glycosylation profiles identical to virion proteins could be determined by the effectiveness of the transmembrane **signal** sequence located at the amino-terminus of premembrane protein. The use of DNA vaccines in multivalent and/or combination vaccines designed to immunize against multiple flaviviruses is also a promising area of development.

L8 ANSWER 3 OF 3 MEDLINE on STN
94201380. PubMed ID: 7512096. An integrated target sequence and **signal** amplification assay, reverse transcriptase-PCR-enzyme-linked immunosorbent assay, to detect and characterize flaviviruses. **Chang G J;** Trent D W; Vorndam A V; Vergne E; Kinney R M; Mitchell C J. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522.) Journal of clinical microbiology, (1994 Feb) 32 (2) 477-83. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.
AB We previously described a reverse transcriptase-PCR using flavivirus genus-conserved and virus species-specific amplimers (D. W. Trent and G. J. Chang, p. 355-371, in Y. Becker and C. Darai; ed., Frontiers of Virology, vol. 1, 1992). Target amplification was improved by redesigning the amplimers, and a sensitive enzyme-linked immunosorbent assay (ELISA) technique has been developed to detect amplified digoxigenin (DIG)-modified DNA. A single biotin motif and multiple DIG motifs were incorporated into each amplicon, which permitted amplicon capture by a biotin-streptavidin interaction and detection with DIG-specific antiserum in a colorimetric ELISA. We evaluated the utility of this assay for detecting St. Louis encephalitis (SLE) viral RNA in infected mosquitoes and dengue viral RNA in human serum specimens. The reverse transcriptase-PCR-ELISA was as sensitive as isolation of SLE virus by cell culture in detecting SLE viral RNA in infected mosquitoes. The test was 89% specific and 95 to 100% sensitive for identification of dengue viral RNA in serum specimens compared with isolation of virus by Aedes albopictus C6/36 cell culture and identification by the indirect immunofluorescence assay.

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L1               2 S E4
L2             825 S (JEV OR JAPANESE ENCEPHALITIS VIRUS)
L3             144 S L2 AND (SIGNAL SEQUENCE)
L4              80 S L3 AND AY<2002
L5              19 S L4 AND SIGNAL/CLM
L6              18 S L5 NOT L1

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L7              50 S E2
L8               3 S L7 AND SIGNAL

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         48297 JAPANESE
         28065 ENCEPHALITIS
        403241 VIRUS
          1049 JAPANESE ENCEPHALITIS VIRUS
                 (JAPANESE(W)ENCEPHALITIS(W)VIRUS)
L9          1101 (JEV OR JAPANESE ENCEPHALITIS VIRUS)

=> s l9 and (signal sequence)
        273291 SIGNAL
        709251 SEQUENCE
          5627 SIGNAL SEQUENCE
                 (SIGNAL(W)SEQUENCE)
L10            3 L9 AND (SIGNAL SEQUENCE)

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L10 ANSWER 1 OF 3 MEDLINE on STN
TI Protective efficacy of a plasmid DNA encoding Japanese encephalitis
   virus envelope protein fused to tissue plasminogen activator signal
   sequences: studies in a murine intracerebral virus challenge model.

L10 ANSWER 2 OF 3 MEDLINE on STN
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TI Expression and secretion of **Japanese encephalitis virus** nonstructural protein NS1 by insect cells using a recombinant baculovirus.

L10 ANSWER 3 OF 3 MEDLINE on STN
TI Analysis of Japanese encephalitis (JE) virus genome and implications for recombinant JE vaccine.

=> d l10,cbib,ab,1-3

L10 ANSWER 1 OF 3 MEDLINE on STN
2002200336. PubMed ID: 11858863. Protective efficacy of a plasmid DNA encoding **Japanese encephalitis virus** envelope protein fused to tissue plasminogen activator signal sequences: studies in a murine intracerebral virus challenge model. Ashok Mundrigri S; Rangarajan Pundi N. (Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India.) Vaccine, (2002 Feb 22) 20 (11-12) 1563-70. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.
AB We report the construction of chimeric DNA vaccine vectors in which secretory **signal sequence** derived from tissue plasminogen activator (TPA) was fused to the full length (pCMVTE) or 398 amino terminal amino acids (pCMVTdeltaE) of **Japanese encephalitis virus** (**JEV**) envelope (E) protein. Transfection studies indicate that E protein expressed from pCMVTdeltaE-transfected cells but not pCMVTE-transfected cells is secreted into the culture medium. Analysis of the potency of various DNA vaccine constructs in a murine intracerebral (i.c.) **JEV** challenge model indicates that pCMVTdeltaE confers the highest level (71%) of protection. Immunization with pCMVTdeltaE induces a mixed Th1 and Th2 T helper cell response while immunization with plasmids encoding nonsecretory forms of E protein induces a Th1 T helper response. Only low levels (<1:20) of virus neutralizing antibody titres were observed in DNA vaccinated mice which did not increase further after i.c. **JEV** challenge. Thus, immunization with a plasmid encoding secretory E protein results in an altered cytokine response and better protection against i.c. **JEV** challenge than that conferred by immunization with plasmids encoding nonsecretory forms of E protein. We also demonstrate that unlike peripheral **JEV** challenge, i.c. **JEV** challenge does not result in an increase in anamnestic antibody response suggesting that other components of immune system such as cytotoxic T cells and T helper cells contribute to protection against i.c. **JEV** challenge of DNA vaccinated mice.

L10 ANSWER 2 OF 3 MEDLINE on STN
93079885. PubMed ID: 1448926. Expression and secretion of **Japanese encephalitis virus** nonstructural protein NS1 by insect cells using a recombinant baculovirus. Flamand M; Deubel V; Girard M. (Laboratoire des Arbovirus, Institut Pasteur, Paris, France.) Virology, (1992 Dec) 191 (2) 826-36. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.
AB The nonstructural protein NS1 of **Japanese encephalitis virus** (**JEV**) was expressed at a high level under the control of the polyhedrin promoter in Spodoptera frugiperda (Sf9) insect cells using a recombinant baculovirus. Recombinant NS1 was designed to contain its natural **signal sequence** at its N-terminus and no C-terminal hydrophobic domain that could act as a membrane anchor. This recombinant protein exhibited similar size to native NS1 expressed in Aedes albopictus (C6/36) insect cells infected with wild-type **JEV**. The **signal sequence** of NS1 allowed translocation of the protein into the endoplasmic reticulum where it underwent glycosylation. A small fraction of synthesized NS1 was able, in the absence of any other viral protein, to associate as a homodimer, showing similar characteristics to the native dimer. Interestingly, this recombinant dimeric form seemed to be exported and released in the extracellular medium of infected cell culture. During its transport, one of the two N-linked oligosaccharides of the polymannose type was processed to an endoglycosidase H-resistant form, suggesting that the protein had passed through the Golgi compartment before reaching the cell surface. Moreover, Triton X-114 partitioning analysis showed that monomeric NS1 behaved essentially as a hydrophilic protein, whereas both intracellular and extracellular dimeric NS1 were either free of or associated to membraneous components.

L10 ANSWER 3 OF 3 MEDLINE on STN
91280411. PubMed ID: 1711716. Analysis of Japanese encephalitis (JE) virus genome and implications for recombinant JE vaccine. Yasui K; Miyamoto M; Kimura-Kuroda J; Yasuda A; Matsuura Y; Sato T; Kojima A; Kubonoya H. (Department of Microbiology, Tokyo Metropolitan Institute for Neurosciences, Japan.) Southeast Asian journal of tropical medicine and public health, (1990 Dec) 21 (4) 663-9. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB From the information of nucleotide sequences and deduced amino acid sequences of flaviviruses including **JEV**, we can postulate processing mechanisms of a polyprotein translated from single long open reading frame of the genome and mechanisms of construction of antigenic structures of structural proteins with biologically active forms after these proteins are translated. The results of comparative analysis of amino acid sequences among flaviviruses and epitope analysis on the E proteins which are the most important antigens for protective immunity suggest that the E protein of flaviviruses may have a similar structure closely related to each other. PrM and E proteins which had predictable signal sequences upstream on the N terminals were expressed with antigenically active form and molecular size the same as the authentic ones by the recombinant viruses. However, the recombinant viruses which had no such **signal sequence** expressed unprocessed proteins with antigenically denatured forms. These results suggest that normal proteolytic processing is needed to construct biologically active structures of **JEV** structural proteins. The E proteins which were expressed by the recombinant viruses as antigenically active form could elicit nutralizing and HI antibodies in animals and protective immunity in mice. The recombinant vaccinia viruses which express the E protein could induce strong immunologic memory against the E protein in mice. These results indicate that the development of a new type of vaccine against **JEV** will become possible in future.

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                E CHANG G J J/AU
L7           50 S E2
L8            3 S L7 AND SIGNAL
L9         1101 S (JEV OR JAPANESE ENCEPHALITIS VIRUS)
L10           3 S L9 AND (SIGNAL SEQUENCE)

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
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